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Mineralocorticoid effect on K^+ permeability of the rabbit cortical collecting tubule

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Mineralocorticoid effect on K^+ permeability of the rabbit cortical collecting tubule. Mineralocorticoid hormones stimulate Na^+ absorption and K^+ secretion by the cortical collecting tubule. There is good evidence that this stimulation involves increasing luminal membrane Na^+ permeability and the turnover rate (or number) of the Na^+-K^+ pumps. These experiments were designed to examine whether mineralocorticoid hormones also increase cell K^+ permeability. Using ^{42}K tracer measurements in tubules treated with amiloride to inhibit active Na^+ and K^+ transport, passive K^+ permeation increased with increasing mineralocorticoid effect. Net Na^+ absorption and the (passive) K^+ efflux rate coefficient (K_K) showed a linear relationship. The stimulatory effect was evident in vitro since 0.2 μM aldosterone added to the bath of tubules harvested from NaCl-loaded rabbits increased K_K at 3 hrs while time controls showed no change. Since these tubules were also treated with amiloride, this increase in K_K was not dependent on increasing Na^+ absorption. The results indicate that in addition to the well-described effects of aldosterone on Na^+ permeability and cell metabolism, the mineralcorticoid effect includes an increase in cellular K^+ permeability.

Effet des minéralocorticoïdes sur la perméabilité au K^+ du tubule collecteur cortical de lapin. Les hormones minéralocorticoïdes stimulent l'absorption de Na^+ et la sécrétion de K^+ par le tubule collecteur cortical. Des preuves solides indiquent que cette stimulation met en jeu une augmentation de la perméabilité membranaire luminale au Na^+ et la vitesse de renouvellement (ou le nombre) de pompes Na^+-K^+. Ces expériences ont étudié si les hormones minéralocorticoïdes augmentent la perméabilité cellulaire au K^+ également. En utilisant des mesures avec un traceur ^{42}K dans des tubules traités par de l'amiloride pour inhiber le transport actif du Na^+ et du K^+, la perméabilité passive au K^+ a augmenté en même temps que l'augmentation de l'effet minéralocorticoïde. L'absorption nette de Na^+ et le coefficient d'efflux de K^+ (passif) (K_K) présentaient une interrelation linéaire. L'effet stimulant était évident in vitro, puisque 0,2 μM d'aldostérone ajoutés au bain des tubules provenant de lapins surchargés en NaCl a augmenté K_K à 3 hrs, alors que les contrôles de temps ne changeaient pas. Puisque les tubules étaient également traîtés par l'amiloride, cette élévation de K_K n' était pas dépendante d'une augmentation de l'absorption de Na^+. Ces résultats indiquent qu'en plus des effets bien décrits de l'aldostérone sur la perméabilité du Na^+ et sur le métabolisme cellulaire, l'effet minéralocorticoïde comporte une augmentation de la perméabilité cellulaire au K^+.

Foremost among the actions of mineralocorticoid hormones is antinatriuresis and kaliuresis. This renal response is effected primarily by the late distal convoluted tubule [1–3] and by the cortical collecting tubule (CCT). Evidence that the CCT is a major target for mineralocorticoid hormone has been obtained using isolated segments for functional studies, aldosterone binding studies, and changes in enzyme activities. There is good agreement that increasing mineralocorticoid hormone activity in vivo increases transepithelial voltage (V_T), as well as Na^+ and K^+ transport in CCT segments harvested from the animal and studied in vitro [4–9]. Aldosterone binds avidly to CCT cells with a high degree of specificity, and in concentrations that are reasonable for those found circulating under physiologic conditions [10–13]. Enzymes whose apparent activity is increased by mineralocorticoid hormone include Na^+-K^+ ATPase [14–18] and citrate synthase [19].

The mechanisms of Na^+ absorption and K^+ secretion by the CCT have been well studied and there is general agreement regarding the pathways involved in the transport of these ions [9, 20–22]. Aldosterone increases Na^+ absorption by at least three mechanisms: *1)* increasing Na^+ permeability of the apical membrane [23–28]; *2)* inducing enzymes involved in intermediary metabolism such as citrate synthase [19, 29], and increasing the activity of Na-K ATPase [14–18]. This latter effect is believed to be secondary to increased Na^+ entry since amiloride can block the increase in activity [17].

There is reason to suspect that mineralocorticoid hormone might increase K^+ permeability. In the distal convoluted tubule, Wiederholt et al [2], demonstrated an increase in the K^+ diffusion voltage following aldosterone administration to adrenalectomized rats. In the rabbit CCT, desoxycorticosterone acetate (DOCA) pretreatment causes both Na^+ absorption (J_{Na}^{net} and K^+ secretion (J_K^{net}) to increase [5, 6, 8]. Of particular interest is that DOCA usually reduces the ratio of these transport rates, J_{Na}^{net}/J_K^{net}, so that for any given increment in Na^+ absorption there is a relatively greater increase in K^+ secretion. These results can be explained by postulating that in addition to increasing apical Na^+ permeability, DOCA also increases apical K^+ permeability. In the toad skin, aldosterone probably increases basolateral K^+ permeability [23].

The present experiments were designed to examine whether the action of mineralocorticoid hormone on the CCT also includes an increase in (passive) cell K^+ permeability. With amiloride in the lumen, the tracer K^+ flux from lumen to bath traverses primarily a cellular pathway [30–32]. Based on these previously determined tracer measurements, we reasoned that differences in tracer flux after mineralocorticoid stimulation would most likely represent differences in cell permeation. The

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results demonstrate that mineralocorticoid hormones increase a passive, cellular K^+ permeation.

Methods

Isolated segments of rabbit CCT were perfused in vitro by methods described previously [33, 34]. Female New Zealand white rabbits weighing 1.2 to 3.0 kg were decapitated, the kidneys removed, and 1 to 2 mm transverse slices dissected in modified Ringer's solution which contained (in mM) NaCl, 105; KCl, 5; $NaHCO_3$, 25; NaH_2PO_4, 2.3; Na acetate, 10; $MgSO_4$, 1; $CaCl_2$, 1.8; glucose, 8.3; and L-alanine, 5. This solution was gassed with 5% CO_2 and 95% O_2 and was used to bathe and perfuse the tubules unless otherwise specified. To reduce adherence of the tubule to glass, bovine calf serum (5% vol/vol) was added to the dissection medium.

All tubules were dissected at 4°C from medullary rays and all segments were obtained from sections of the medullary ray superficial to the cortical-medullary junction.

After dissection, the tubules were transferred to a plastic chamber, the two ends mounted in glass pipettes, and a concentric perfusion pipette was advanced into the lumen for a distance of 75 to 100 μm. This perfusion pipette served as the voltage sensing electrode. All tubules were equilibrated at 37°C for 60 min or until the V_T was stable, whichever was longer. The bath was changed continuously at a rate of 0.5 ml/min. Circuitry for the voltage measurements was as described previously [34]. Voltages are reported using bath as ground. After V_T had stabilized and initial measurements made, the perfusate was changed to one that contained 0.1 mM amiloride (Merck, Sharp, and Dohme, West Point, Pennsylvania, USA). The change in V_T from a negative value to a slightly positive value (the amiloride-sensitive V_T) was taken as an index of active Na^+ absorption. All subsequent maneuvers were conducted with amiloride in the lumen.

The perfusion solution contained [*methoxy*-^{3}H] inulin, exhaustively dialyzed to remove substances of molecular weight less than 3000. Volume absorption (J_v) was calculated for each period using the standard expression [34]. No period was accepted if J_v was more than ± 0.1 nl/mm/min. ^{22}Na and ^{42}K were added to the perfusate to obtain concentrations of approximately 15 and 40 μCi/ml, respectively. Because the specific activity of ^{42}K (New England Nuclear, Boston, Massachusetts, USA) was variable, the actual [K^+] of the perfusate was measured by flame photometry. By using high specific activity material, total [K^+] was never greater than 7.0 mM and was usually between 5.0 and 6.0 mM.

Lumen to bath fluxes of ^{22}Na and ^{42}K were calculated by measuring the difference between perfused and collected concentrations of the tracer. Perfusion rate was maintained between 4 and 9 nl/min by varying hydrostatic pressure. The reported unidirectional fluxes are the mean of 3 to 5 periods. The flux (for K) is reported as a rate coefficient (K_K, nm/s) calculated by the following expression:

$$K_K = \frac{2\ V_L}{A}\left[\frac{C_o^* - C_L^*}{C_o^* + C_L^*}\right]$$

where V_L is the collection rate ($cm^3 \cdot sec^{-1}$); A, the area of the luminal surface of the tubule (cm^2) assuming a diameter of 25 μm, and C_o^* and C_L^* the activity of the tracer in the perfused and collected fluid, respectively. The major assumption underlying this expression is that the changes in the specific activity of the perfusate along the tubule are insufficient to cause significant differences in the calculated K_K if the transport is carrier mediated. For this reason, the C_L^*/C_o^* was maintained above 0.75 in most cases. The derivation of this expression has been presented previously [8]. The Na^+ efflux (J_{Na}) was calculated using the more traditional units of pEq/mm · min. Corrections were made for ^{42}K decay during the counting process.

Blood was collected at the time of sacrifice in heparinized containers and the plasma frozen for later serum aldosterone determination by radioimmunoassay. Steroids were extracted from 4 ml of plasma with 40 ml methylene chloride and aldosterone was separated using Sephadex LH-20. The reconstituted extract was assayed for aldosterone by radioimmunoassay using a specific antibody. The antibody, ^{3}H-aldosterone, and the aldosterone standard were purchased from New England Nuclear. Unbound aldosterone was adsorbed onto dextran-coated charcoal. Fractional recovery for the process ranged from 50 to 60%. Coefficient of variation for a single serum sample was less than 20%.

Statistical analysis was performed by paired or unpaired *t* test or by the Mann-Whitney test as indicated in the text.

Results

Lumen-to-bath fluxes of ^{22}Na and ^{42}K were conducted simultaneously both before and after amiloride (0.1 mM in the lumen). The results of 12 experiments are displayed in Table 1. Five rabbits were pretreated with DOCA from 1 to 5 days to extend the range of Na^+ absorption [22]. Amiloride reduced the lumen-to-bath Na^+ flux (J_{Na}) to 10.5 ± 1.5 pEq/mm · min (corresponding to a rate coefficient of 17.8 ± 2.5 nm/s). In every tubule, K_K increased, as has been reported previously [30].

From these data, the relationships between Na^+ absorption and K^+ permeability can be expressed in two ways. First, the values of J_{Na} and K_K measured before amiloride can be compared. The pre-amiloride value for J_{Na} is a modest overestimation of active Na^+ transport since the passive component is relatively small and constant. The relationship between these two variables is displayed in Figure 1 and appears to be correlated. Second, the post-amiloride K_K can be plotted as a function of the amiloride-sensitive J_{Na} (net Na^+ transport). This relationship is displayed in Figure 2. DOCA pretreatment increased mean net Na^+ absorption by a factor of 2.9 from 24.8 ± 4.2 pEq/mm · min in tubules from normal rabbits to 72.9 ± 9.7 pEq/mm · min in tubules from DOCA-treated rabbits. K_K was increased by a factor of 3.1 (from 201 ± 30 to 621 ± 95 nm/s). Both increases are highly significant ($P < 0.001$ by unpaired analysis). When the values for each tubule are analyzed, it appears that they are highly correlated. Linear regression analysis places the intercept near the origin.

In vitro effect of aldosterone

The effect of DOCA on K_K was sufficiently large that it seemed reasonable to test the possibility that in vitro aldosterone might also increase K_K. Because previous efforts to stimulate ion transport in CCT with in vitro aldosterone have met with mixed success [4, 5] a slightly modified preparatory protocol was used for these rabbits. Each rabbit was given 0.9% NaCl solution to drink for 4 or 5 days before sacrifice. The

Table 1. Effect of amiloride on Na^+ and K^+ transport across CCT

	Control			0.1 mM amiloride			
Experiment	V_T *mV*	J_{Na}^{lb} *pEq/mm · min*	K_K *nm/s*	V_T *mV*	J_{Na}^{lb} *pEq/mm · min*	K_K *nm/s*	DOCA *days*
1	−15	21.8	40	+2	7.6	83	0
2	−11	26.1	74	−1	9.2	150	0
3	−47	48.0	72	+3	13.6	326	0
4	−20	29.8	69	+1	12.9	208	0
5	−28	41.6	86	+1	4.3	267	0
6	−34	31.1	55	−3	2.4	196	0
7	−16	37.5	66	+5	13.7	178	0
8	−39	66.0	70	+6	3.3	318	2
9	−38	94.4	227	0	15.3	733	4
10	−37	65.8	81	0	10.9	518	5
11	−30	78.4	307	−1	18.1	658	1
12	−38	115.7	211	−1	14.9	876	3

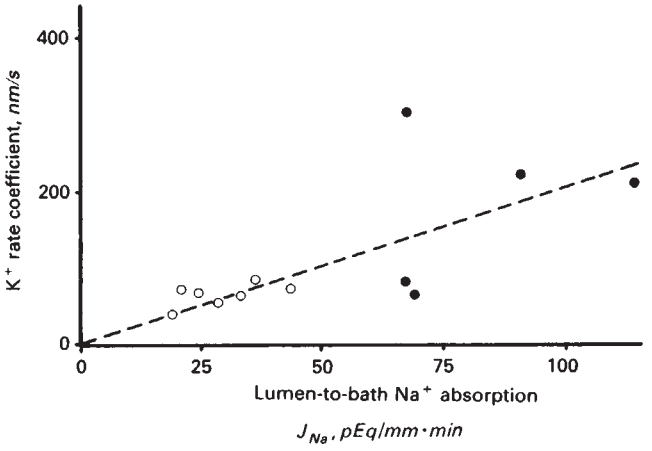

Fig. 1. *Relationship between unidirectional Na^+ absorption and K_K (lumen-to-bath K^+ rate coefficient) measured during active transport.* Symbols are: •, DOCA-treated; ○, normal. Linear regression equation is y = 2.23 × −9.1, r = 0.78.

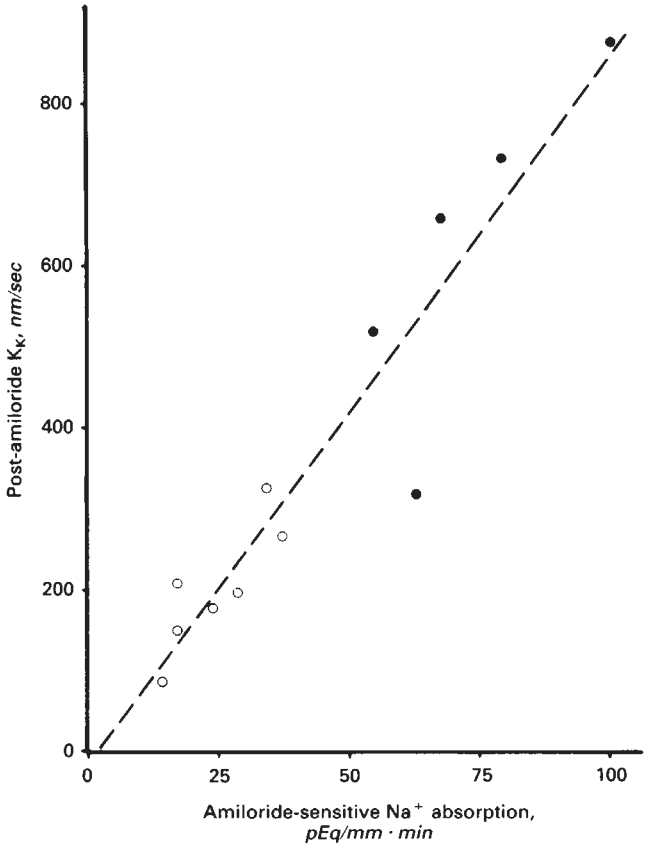

Fig. 2. *Relationship between amiloride-sensitive Na^+ absorption and K_K measured after inhibition of active transport with amiloride.* Symbols same as Fig. 1. Linear regression equation is y = 8.74 × −15.5, r = 0.95.

intent was to suppress endogenous aldosterone production while not affecting production of other hormones synthesized by the adrenal gland, as would be the case with adrenalectomy. As an estimate of the validity of this assumption, plasma aldosterone concentrations were determined at the time of sacrifice. For comparison, measurements were also made on a group of DOCA-treated rabbits and a group of normal rabbits. As shown in Table 2, plasma aldosterone in normal rabbits was significantly higher than in DOCA-treated or NaCl-treated rabbits. Consistent with suppression of endogenous aldosterone by DOCA, there was no difference between groups treated with DOCA and NaCl.

Tubules were treated with 0.1 mM amiloride (in the lumen) to eliminate net Na^+ and K^+ transport. Seven tubules were exposed to 0.2 μM aldosterone (in the bath) after measuring the control values for K_{Na} and K_K, and four tubules were exposed only to the appropriate concentration of the ethanol carrier after the control period. The results are depicted in Table 3. The time control experiments showed no change in K_{Na} or K_K while these values increased in the aldosterone-exposed group. The increase in K_K was variable and in the aggregate, rather modest. The increase was statistically significant ($P < 0.05$) by paired analysis. Evaluation by the non-parametric Mann Whitney test revealed that the two groups were different ($P < 0.05$). V_T was unchanged in both groups.

The increase in K_K after aldosterone exposure was accompanied by an increase in K_{Na}, although the mean increase in K_K was substantially larger than K_{Na} (72 ± 34 nm/s vs. 5.3 ± 0.9 nm/s).

In three of the tubules treated with aldosterone, a fourth experimental period was added where 0.5 mM ouabain was

Table 2. Plasma aldosterone values for rabbits at sacrifice

	Normal	DOCA-treated	Saline-treated
Aldosterone, *ng/ml*	0.303* ± 0.032	0.146 ± 0.027	0.158 ± 0.017
N	13	5	14

Values are mean ± SEM.
* Value significantly larger than other two groups by analysis of variance ($P < 0.05$).
DOCA-treated rabbits received 5 mg daily for 1 to 4 days.
Saline-treated rabbits received 0.9% NaCl drinking water for 4 to 5 days.

Table 3. Effect of in vitro aldosterone on K_K

			0.2 μM aldosterone in bath			
	Control		90 to 120 min		150 to 180 min	
	K_{Na}	K_K	K_{Na}	K_K	K_{Na}	K_K
Experiment	*nm/s*		*nm/s*		*nm/s*	
1	−3.9	229	—	—	2.8	342
2	9.2	105	17.0	128	16.6	132
3	10.5	242	16.9	379	15.7	491
6	22.4	123	19.9	159	25.6	167
7	6.4	186	4.9	166	7.6	150
9	4.5	181	10.8	210	11.9	218
11	4.2	176	2.2	209	9.9	246
Mean	7.6	177	11.9	209	12.9*	249*
± SEM	± 3.0	± 19	± 2.9	± 36	± 2.8	± 48
			Time Controls			
4	11.5	132	5.3	132	3.5	107
5	28.6	149	21.0	152	24.8	137
8	10.6	125	8.9	165	12.8	161
10	13.6	277	17.4	231	18.6	211
Mean	16.1	171	13.1	170	14.9	154
± SEM	± 4.2	± 36	± 3.6	± 21	± 4.5	± 22

0.1 mM amiloride present continuously in the lumen.
* $P < 0.05$ compared to control value by paired analysis.

Table 4. Effect of ouabain on K_{Na} and K_K after in vitro exposure to aldosterone

	0.2 μM aldosterone *150 to 180 min*		Aldosterone + ouabain *0.5 mM*	
Experiment	K_{Na}	K_K	K_{Na}	K_K
7	7.6	150	1.9	12.1
9	11.9	218	9.7	26.1
11	9.9	246	8.2	23.1

added to the bath. Previous results have demonstrated that ouabain reduces the K^+ permeability in the amiloride-treated CCT [30]. These experiments were conducted to see whether in vitro aldosterone would mitigate this effect. In the presence of aldosterone, ouabain reduced K_K from 205 to 20 nm/s (Table 4). These reductions in K_K are similar to those reported for tubules not exposed to in vitro aldosterone [31] and indicate that in vitro aldosterone had little or no effect on this response.

Discussion

These experiments demonstrate that, in addition to its well-described effect on increasing Na^+ absorption by the cortical collecting tubule, mineralocorticoid hormone increases cellular K^+ permeability. This effect is most clearly displayed in Figure 2 where the tracer K^+ permeation in the absence of net transport (amiloride-treated) is highly correlated to the mineralocorticoid state of the tubule. These experiments also show that in vitro exposure of CCT to a high concentration of aldosterone for 3 hrs increases K^+ permeability (Table 3).

Because amiloride inhibits K^+ secretion [6, 35] and reduces V_T to a small positive value (Table 1), K_K is a measure of passive transport. Previously reported experiments indicate that this flux is predominantly cellular [30, 31], and is thus determined by the magnitude of transport across both apical and basolateral membranes. The nature of K^+ permeation across these membranes is not completely understood at the present time. However, conductive permeation plays a significant role in the apical membrane and very probably in the basolateral membrane. These deductions were initially made using transepithelial measurement [21, 32, 36] and have recently been supported by intracellular impalements [9, 37]. Most recently, the apical K^+ channel has been demonstrated using the patch clamp technique [38, 39]. Thus, present evidence indicates that, at least in part, cellular K^+ permeation is conductive. It is thus reasonable to presume that mineralocorticoid hormones increase this conductive pathway.

The idea that aldosterone may play an important role in regulating K^+ permeability of the distal convoluted tubule has been advanced by Wiederholt et al [2, 3] using K^+ diffusion V_T measurements. Additional data have been presented by Hierholtzer et al [1] who demonstrated that the late distal tubule of adrenalectomized rats had an impaired ability to generate a K^+ concentration gradient. Taken together, these data are consistent with the notion that aldosterone increases conductive K^+ permeability of the apical membrane of the late distal convoluted tubule.

The mechanism whereby mineralocorticoid hormone causes an increase in cell K^+ permeability is not clear. Aldosterone exerts its effects by a process that generally requires at least 90 min to become evident. The experiments examining the effect of in vitro aldosterone exposure to CCT harvested from animals having received 0.9% NaCl to drink were designed to assess four aspects of the mechanism: *a)* is there an in vitro effect; *b)* what is the time course; *c)* is there an effect in the absence of net Na^+ transport; and *d)* is the increase in permeability selective for K^+?

In vitro stimulation of CCT ion transport by aldosterone has proven difficult to establish [4, 5, 40]. Although the experiments reported in Table 3 display some inconsistency regarding in vitro aldosterone effects, in the aggregate, K^+ permeability was significantly increased at 3 hrs. The onset of action was apparently between 60 and 180 min. This onset of action follows the general kinetic pattern of steroid hormone action.

The in vitro aldosterone experiments were conducted in the presence of amiloride to examine the question of whether K^+ permeability would be increased in the absence of Na^+ transport. This question focuses on the issue of whether or not the effect is an event stimulated by aldosterone primarily or whether it is secondary to a possible increase in Na^+ transport.

An example of a secondary effect of aldosterone is the increase in Na^+-K^+ ATPase activity which occurs following aldosterone exposure. Pretreatment with amiloride to prevent Na^+ entry through the apical membrane prevents the increase in activity [17]. The increase in K^+ permeability in the present experiments (Table 3) demonstrates that the acute increase is not secondary to increasing net Na^+ absorption, but may be another primary effect of mineralocorticoid hormones.

The selectivity of the increase in K^+ permeability was examined by measuring the efflux of ^{22}Na simultaneously. Somewhat surprisingly, aldosterone increased K_{Na} (Table 3). This small increase can be explained in one or more of the following ways: *a)* The increase could have been secondary to a modest increase in the permeability of the paracellular pathway. *b)* The exposure to aldosterone might have reduced the affinity of the Na^+ channel for amiloride such that Na^+ could gain entry via that pathway more readily [25]. *c)* Finally, it might have been owing to an increase of Na^+ permeation through K^+ channels. If the increase in K_{Na} were entirely via this mechanism then the K^+:Na^+ selectivity of the induced pathway would be ($\Delta K_K/\Delta K_{Na}$) approximately 12.9. This value is similar to the relative selectivity for K^+ and Na^+ of 14.3 in the delayed rectifier of the snail neuron [41].

Although the reason for the increase in K_{Na} is not clear, the increase in K_K was not owing to an increase in non-selective permeability of the tubule. If such were the case, the ratio of the change in K^+ permeation to Na^+ permeation should be closer to 1.5 (the ratio of their mobilities in water) rather than 12.9. Additional evidence against this possibility is contained in the experiments where Na^+-K^+ ATPase was inhibited with ouabain (Table 4). This maneuver has previously been shown to reduce cell K^+ permeability [31]. The selective reduction in K_K demonstrates that aldosterone exposure does not alter this regulatory mechanism. Thus, the aggregate evidence indicates that aldosterone stimulated a K^+-selective, cellular pathway.

Conclusions regarding whether mineralocorticoid hormone increases K^+ permeation at the apical or basolateral membrane of both cannot be made with certainty. In this regard, Koeppen and Giebisch [42] have evidence that mineralocorticoid hormone increases both apical and basolateral K^+ conductances.

The significance of this increase in cell K^+ permeability might be summarized as follows. Aldosterone, by increasing Na^+ absorption, increases the lumen-negative V_T with a cell voltage profile favoring K^+ secretion. An increase in apical membrane (conductive) K^+ permeability, together with a more favorable electrical gradient, would enhance K^+ secretion. Such a scenario would explain why K^+ secretion increases to a greater extent than does Na^+ absorption following DOCA treatment [5, 6, 8]. An interesting possibility emerges from the notion that both apical and basolateral membranes increase K^+ permeability after aldosterone. Koeppen, Biagi, and Giebisch [9] have determined that the basolateral membrane voltage of DOCA treated rabbits is −106 mV (compared to the normal value of −84 mV). The electrical gradients would thus favor K^+ entry across the basolateral membrane as well as K^+ secretion across the apical membrane. An increase in both apical and basolateral membrane conductive permeabilities would enhance passive K^+ secretion via a cellular pathway. Thus, conceivably the magnitude of net K^+ secretion would not be limited by the rate of K^+ entry via the Na^+-K^+ pump.

It is likely that the increase in cell permeability is, like the increase in apical Na^+ permeability and the induction of citrate synthase, a primary effect. This regulatory influence on cell K^+ permeability is consistent with its role in long-term regulation of K^+ secretion. The mechanism(s) whereby increased K^+ permeability is effected and the precise nature of this permeation remain fruitful areas for investigation.

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