Characterization of Aldosterone-induced Potassium Secretion in Rat Distal Colon

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Abstract

The role of apical and basolateral membranes in aldosterone-induced active potassium (K) secretion in rat distal colon was investigated by measuring mucosal-to-serosal (J_{ms}) and serosal-to-mucosal (J_{sm}) ^{42}K fluxes ($\mu eq \cdot h^{-1} \cdot cm^{-2}$) across isolated stripped mucosa under short-circuit conditions in normal and secondary-hyperaldosterone animals. In normal colons mucosal tetraethylammonium (TEA; 30 mM) or barium (Ba; 5 mM), but not cesium (Cs; 15 mM), reduced J_{sm} without affecting J_{ms}. In aldosterone animals (*a*) net K secretion (-0.54 ± 0.11) was converted to net K absorption (0.63 ± 0.15) by mucosal TEA, which produced a marked reduction in J_{sm} (0.82 ± 0.07) and an increase in J_{ms} (0.35 ± 0.07). In contrast mucosal Ba resulted in a relatively smaller reduction in J^K_{sm} without altering J^K_{ms}, whereas mucosal Cs was ineffective; (*b*) serosal bumetanide or the removal of serosal Na or Cl markedly inhibited J^K_{sm} and abolished net K secretion; and (*c*) serosal ouabain (1 mM) produced qualitatively similar effects to those of serosal bumetanide. These results demonstrate that (*a*) normal rat distal colon contains apical TEA- and Ba-sensitive K channels; (*b*) aldosterone induces TEA-sensitive and Ba-sensitive apical K channels; (*c*) aldosterone-induced K secretion requires both the Na,K-pump and Na-K-2Cl cotransport for K uptake across the basolateral membrane; and (*d*) alteration of any of these processes results in inhibition of aldosterone-induced active K secretion simultaneously with stimulation of K absorption.

Introduction

Aldosterone stimulates active potassium (K) secretion in the mammalian colon (1–3). Studies of aldosterone-induced active K secretion in the distal colon of the rat in vitro have demonstrated that this secretory process is Na dependent, Cl dependent, electrogenic, and amiloride insensitive (2), whereas electrophysiologic studies have suggested that aldosterone may induce a tetraethylammonium- (TEA)[1] inhibitable K conductance in the apical membrane (4). The role of this apical channel in K secretion has not been investigated, and it is not known whether this K channel is or can also be inhibited by other putative K channel blockers.

The uptake of K across the basolateral membrane of colonic epithelial cells can be mediated by two distinct transport processes: Na,K-ATPase and Na-K-2Cl cotransport. Hyperaldosteronism results in a significant increase in Na,K-ATPase activity (5) and the induction of electrogenic Na absorption (6). It is most likely therefore that the Na,K-pump is (at least partially) responsible for the increase in K uptake across the basolateral membrane in hyperaldosterone animals. However, Na-K-2Cl cotransport process could also be involved in K secretion. This possibility is not unlikely because serosal bumetanide or furosemide, inhibitors of Na-K-2Cl cotransport, block prostaglandin E_2, epinephrine, and adenosine-induced K secretion in rabbit colon (7, 8) and vasoactive intestinal polypeptide–induced Cl secretion in T_{84} colonic cancer cells (9). Thus, it is not known whether the Na-K-2Cl cotransport contributes to K uptake across the basolateral membrane in aldosterone-induced K secretion, and if so, the relative contribution of the Na,K-pump and Na-K-2Cl cotransport to overall K secretion.

Schultz (see review, reference 10) has emphasized the importance of increased basolateral K conductance during situations in which there are enhanced rates of Na absorption and Na,K-pump activity. Thus, amino acid and sugar transport in the Necturus small intestine is associated with an increase in transepithelial Na absorption and basolateral K conductance (11); the addition of barium (Ba) to the serosal medium decreases both K conductance and Na absorption. Recently, studies by Dharmsathaphorn et al. (9) in T_{84} colon cancer cells have indicated that Ba-sensitive basolateral K channels may be important in cAMP-stimulated Cl secretion. The role, if any, of basolateral K channels in the aldosterone-induced K secretion is unknown.

This study was designed to provide additional characterization of the mechanism of aldosterone-induced active K secretion in isolated distal colonic mucosa of Na-depleted rats. Attention was primarily directed towards identification of the properties of apical and basolateral K channels and the mechanism of K uptake across the basolateral membrane. The results demonstrate that aldosterone-induced K secretion is associated with the stimulation (or induction) of TEA-sensitive and Ba-sensitive K channels in the apical membrane. Furthermore, these experiments establish a role for both Na,K-ATPase and Na-K-2Cl cotransport in aldosterone-induced K secretion by mediating the uptake of K across the basolateral membrane of the distal colon.

Methods

Two groups of nonfasting male Sprague-Dawley rats weighing 220–280 g were used in this study. Group 1 rats were normal animals fed a standard diet (Prolab 3000; Agway Inc., Syracuse, NY) containing 19 meq Na/100 g food and 24 meq K/100 g food. Group 2 consisted of rats fed a paste diet (prepared in our laboratory) to which Na was not added and contained 20 meq K/100 g food. The Na-deficient diet was given for a period of 7–9 d before the experiments to induce secondary hyperaldosteronism; previous studies have demonstrated that such animals have plasma aldosterone levels that are ~ 100-fold

A portion of these studies has been published in abstract form (1988. *J. Physiol.* [*Lond.*]. 396:34P and 1988. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* A748. [Abstr.]).

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1. *Abbreviations used in this paper:* G, conductance; I_{sc}, short-circuit current; J^K_{ms}, mucosal-to-serosal K flux; J^K_{net}, net K movement; J^K_{sm}, serosal-to-mucosal K flux; PD, transmural potential difference; TEA, tetraethylammonium chloride.

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greater than normal animals (12). This group of rats will be referred to as the aldosterone or Na-depleted group.

The procedure has previously been described in detail (6). Approximately 15 min after mounting the last of eight segments, the transepithelial potential difference (PD) was noted, all tissues were then clamped to zero PD and the short-circuit current (I_{sc}) was measured, and the conductance (G) was calculated as previously described (6). The tissues were then paired on the basis of a conductance difference of < 10%.

Transmural fluxes of potassium from mucosa-to-serosa or serosa-to-mucosa were measured under short-circuit conditions using ^{42}K as previously described (2, 6). Under short-circuit conditions, net potassium transport (J^K_{net}) is active and defined as the difference between mucosal-to-serosal flux (J^K_{ms}) and serosal-to-mucosal flux (J^K_{sm}). Positive and negative values of J^K_{net} represent active absorption and active secretion, respectively.

The Ringer's solution contained (in millimolar per liter): NaCl, 115, $NaHCO_3$, 25; K_2HPO_4, 2.4; KH_2PO_4, 0.4; $CaCl_2$, 1.2; $MgCl_26H_2O$, 1.2; and glucose, 10 (pH 7.4). In sodium-free experiments, NaCl and $NaHCO_3$ were replaced by equimolar choline-Cl and choline-HCO_3, respectively. In chloride-free experiments, NaCl was replaced by equimolar Na-isethionate, and $CaCl_2$ and $MgCl_2$ by their respective sulfate salt. In experiments in which the effect of barium was tested, K_2HPO_4 and KH_2PO_4 were replaced by equimolar (5.2 mM) KCl, to prevent precipitation of barium-phosphates. Drugs or other agents were added from concentrated stock solutions to give the desired concentration.

^{42}K was purchased from New England Nuclear (Boston, MA). Bumetanide was a gift from Hoffmann-La Roche, Nutley, NJ. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

All values are expressed as mean±SEM. Statistical analysis was performed using paired (unless otherwise stated) *t* test and a *P* value of ≤ 0.05 was considered significant.

Results

Table I demonstrates that net K absorption was present in normal animals and that secondary hyperaldosteronism converted this net K absorption to net K secretion. These results are in agreement with previous studies from our laboratory (2, 6).

Effect of ion substitution on K secretion. The dependence of K secretion on serosal Na and/or Cl was investigated in Na-depleted animals by completely substituting choline for Na and isethionate for Cl in the Ringer's solution bathing the serosal side. As shown in Table I (section B), the absence of serosal Cl resulted in the conversion of net K secretion to net K absorption as a result of a marked decrease in J^K_{sm} and an increase in J^K_{ms}. The absence of serosal Na resulted in zero net K movement due to significant reduction (41%) in J^K_{sm} and increase (50%) in J^K_{ms}.[2] In contrast, when mucosal and serosal sides were bathed by Na-free Ringer's, J^K_{sm} was reduced to 0.19±0.03 $\mu eq \cdot h^{-1} \cdot cm^{-2}$, a value that probably reflects diffusional K movement, and J^K_{ms} increased to 2.37±0.27 (n = 8), as shown previously (2).

Effect of serosal bumetanide and ouabain. The results obtained in the serosal side ion substitution experiments clearly demonstrate that serosal Na and Cl are required for maximal K secretion in aldosterone animals suggesting a role for a basolateral Na-K-2Cl cotransport process and/or the Na,K-pump. We, therefore, tested the effect of serosal addition of bumetanide or ouabain, inhibitors of the former and latter transport systems, respectively.

In the normal distal colon 1 mM bumetanide significantly inhibited the relatively low J^K_{sm} (0.36±0.04 vs. 0.23±0.02 $\mu eq \cdot h^{-1} \cdot cm^{-2}$, $P < 0.005$). However, because of its small magnitude, the inhibitory effect was not reflected in a change in net K absorption. On the other hand in the Na-depleted animal 1 mM bumetanide produced a marked inhibitory effect on J^K_{sm} (1.40±0.05 vs. 0.43±0.03 $\mu eq \cdot h^{-1} \cdot cm^{-2}$, $P < 0.001$) as well as a marked rise in J^K_{ms} (0.80±0.09 vs. 1.27±0.16 $\mu eq \cdot h^{-1} \cdot cm^{-2}$, $P < 0.005$). Thus, net K secretion was converted to net K absorption (0.83±0.16 $\mu eq \cdot h^{-1} \cdot cm^{-2}$). To test the specificity of the bumetanide effect, experiments were also performed in the Na-depleted group with 10 μM bumetanide (Fig. 1). The effect of the addition of 10 μM bumetanide (Fig. 1) was virtually identical to that observed with 1 mM bumetanide.[3] 10 μM bumetanide also increased the I_{sc} by 1.1±0.3 $\mu eq \cdot h^{-1} \cdot cm^{-2}$ ($P < 0.01$).

Fig. 2 illustrates the results obtained with serosal addition of 1 mM ouabain (a large dose was used because of the known low sensitivity of rat tissues to glycosides) to the distal colon of Na-depleted animals. Ouabain converted net K secretion (−0.40±0.11 $\mu eq \cdot h^{-1} \cdot cm^{-2}$) to a large net absorptive flux (1.36±0.10 $\mu eq \cdot h^{-1} \cdot cm^{-2}$) as a result of a marked inhibition (~ 88%) and augmentation (~ 120%) in the active components of J^K_{sm} and J^K_{ms}, respectively. This was also accompanied by a marked reduction in the I_{sc} from 6.2±0.4 to 1.9±0.2 $\mu eq \cdot h^{-1} \cdot cm^{-2}$ ($P < 0.001$).

The increase in I_{sc} (1.1±0.3 $\mu eq \cdot h^{-1} \cdot cm^{-2}$) in response to bumetanide (10 μM) is consistent with a decrease in electrogenic K secretion as this change was very similar to the change in J^K_{sm} (−0.97 + 0.05 $\mu eq \cdot h^{-1} \cdot cm^{-2}$). In contrast, although serosal ouabain decreased J^K_{sm} by an equivalent amount (−0.95±0.18 $\mu eq \cdot h^{-1} \cdot cm^{-2}$), the I_{sc} was inhibited (by 4.3±0.4 $\mu eq \cdot h^{-1} \cdot cm^{-2}$) (Fig. 2); the latter effect reflected an inhibition of electrogenic Na absorption, which is present in aldosterone animals (6, 13).[4]

Effect of putative K channel blockers. The effects of putative K channel blockers were first studied in the aldosterone group, because in these animals a large K secretory flux was present compared to normal animals (Table I). When a K

2. Because unidirectional K fluxes (J^K_{ms} and J^K_{sm}) represent the sum of active and passive components, results expressed in percentage terms refer to alterations in only the active component. Detailed analysis (Sweiry, J., and Binder, H. J., manuscript submitted for publication) demonstrated that in the absence of Na from mucosal and serosal solutions, J^K_{sm} is a linear function of K concentration; at K = 5.2 mM, J^K_{sm} is 0.22 and 0.16 $\mu eq \cdot h^{-1} \cdot cm^{-2}$ in normal and Na-depleted animals, respectively. These values are not significantly different from those in the present study under similar conditions: J^K_{sm} was 0.22±0.02 and 0.19±0.03 $\mu eq \cdot h^{-1} \cdot cm^{-2}$ in normal (n = 9) and Na-depleted (n = 8) animals, respectively. Thus, the serosa-to-mucosa K flux measured in the absence of mucosal and serosal Na most likely represents diffusional movement of K. The active component of unidirectional K movement is the difference between the isotopically determined unidirectional K movement and the diffusional component.

3. In limited studies in which only J^K_{sm} was determined in aldosterone animals 1 μM bumetanide reduced J^K_{sm} from 1.50±0.07 to 0.92±0.04 $\mu eq \cdot h^{-1} \cdot cm^{-2}$; n = 12.

4. Previous studies (6) have demonstrated that in sodium-depleted animals the I_{sc} can be accounted for by net Na absorption (7.3±0.5 vs. 6.9±0.7 $\mu eq \cdot h^{-1} \cdot cm^{-2}$, respectively). The addition of 10 μM amiloride to the mucosal bathing solution reduced J^{Na}_{net} almost to zero and unmasked electrogenic K secretion whose magnitude was relatively small compared to net Na absorption (between 0.7 and 1.1 $\mu eq \cdot h^{-1} \cdot cm^{-2}$ (2).

Table I. Potassium Fluxes and Associated Electrical Parameters in the Distal Colon of Normal and Na-depleted Rats, and the Effect of Ion Substitution in the Latter Group

	J_{ms}	J_{sm}	J_{net}	I_{sc}	PD	G	n
		$\mu eq \cdot h^{-1} \cdot cm^{-2}$			mV	$mS \cdot cm^{-2}$	
A. Normal							
Ringer's	0.78±0.07	0.41±0.03	0.38±0.05	0.8±0.1	3.8±0.5	5.6±0.4	4
B. Na-depleted							
Ringer's	0.69±0.05	1.38±0.03	−0.69±0.08	6.9±0.7	18.9±2.6	10.2±0.8	
P*	NS	<0.001	<0.001	<0.001	<0.005	<0.005	5
Serosal Na-free	0.94±0.08	0.88±0.08	0.06±0.12	7.5±0.6	25.4±1.4	8.0±0.7	
P‡	<0.05	<0.001	<0.001	NS	<0.05	NS	7
Serosal Cl-free	1.23±0.09	0.61±0.08	0.62±0.07	5.9±0.4	26.4±4.0	6.4±0.7	
P‡	<0.001	<0.001	<0.001	NS	NS	<0.01	5

Values are mean±SE of two 15-min flux periods; n, number of tissue pairs. PD, potential difference (millivolts, serosa positive); G, conductance. J^K_{ms}, mucosal-to-serosal potassium flux; J^K_{sm}, serosal-to-mucosal potassium flux; J^K_{net}, net potassium transport (absorption and secretion are represented by positive and negative values, respectively); I_{sc}, short-circuit current. Unless otherwise stated solutions were normal Ringer's on both mucosal and serosal sides. Sodium and chloride were replaced by choline and isethionate, respectively. P (unpaired t tests) values are: * compared with normal group (A); ‡ compared with Na-depleted group in Ringer's.

channel blocker was found to alter K transport, the same experimental protocol was repeated in the colon of normal animals.

Table II shows the effects of mucosal addition of TEA (30 mM), 5 mM Ba, or 15 mM Cs on unidirectional and net K fluxes across the distal colon of sodium-depleted rats.[5] Mucosal TEA reversed net K secretion (-0.54 ± 0.10 $\mu eq \cdot h^{-1} \cdot cm^{-2}$) observed in the aldosterone group to net K absorption (0.63 ± 0.15 $\mu eq \cdot h^{-1} \cdot cm^{-2}$), as a result of both a marked decrease in J^K_{sm} and an increase in J^K_{ms}. Mucosal Ba abolished net K secretion (-0.67 ± 0.11 vs. 0.03 ± 0.1 $\mu eq \cdot h^{-1} \cdot cm^{-2}$) mainly as a result of a reduction in J^K_{sm}. Compared with TEA, the inhibition of J^K_{sm} by Ba was smaller (0.54 ± 0.01 vs. 0.82 ± 0.07 $\mu eq \cdot h^{-1} \cdot cm^{-2}$, respectively, $P < 0.05$). Mucosal Cs did not alter unidirectional K fluxes.

Table III shows the results obtained with serosal addition of TEA, Ba, or Cs in the aldosterone group. Serosal TEA was ineffective in inhibiting either unidirectional K fluxes, and serosal Ba only marginally reduced J^K_{ms} without a significant effect on any of the parameters measured. Serosal Cs, however, produced a marked effect on unidirectional and net K fluxes; J^K_{net} was reversed from net secretion to net absorption as a consequence of an ~ 83% decrease in J^K_{sm} and ~ 144% increase in J^K_{ms}. In normal animals serosal Cs did not affect J^K_{ms}: 0.74 ± 0.10 vs. 0.78 ± 0.09 $\mu eq \cdot h^{-1} \cdot cm^{-2}$, but J^K_{sm} was significantly reduced: 0.56 ± 0.07 vs. 0.27 ± 0.02 $\mu eq \cdot h^{-1} \cdot cm^{-2}$ ($P < 0.005$). To distinguish whether the effect of Cs was due to an inhibition of a basolateral K channel or an effect on basolateral Na-K-2Cl cotransport that exists in this tissue (see above), experiments were conducted in the absence of Cl from the serosal solution. Under these conditions the cotransport system would not be functional and any effect of serosal Cs would be due to either competition for K uptake via the Na,K-pump or an effect on basolateral K channel. The results (Table III [section D]) demonstrate that although J^K_{sm} was significantly reduced, the absorption of Na (represented by the I_{sc}) was unaffected and therefore, the Na,K-pump was still operative. Thus, Cs affects active K secretion primarily by inhibiting Na-K-2Cl cotransport system; the Na,K-ATPase appears to have an affinity for Cs resulting in a reduction in K uptake but without affecting pump activity.

The putative K channel blockers that were effective in blocking K fluxes across colonic mucosa of the aldosterone group, namely mucosal TEA or Ba, were further investigated to determine their effect on K transport in the colon of the normal rat (Fig. 3). TEA reduced the active component of J^K_{sm} by 100% to a value (0.21 ± 0.02 $\mu eq \cdot h^{-1} \cdot cm^{-2}$) representative of diffusional transfer[2] but, because of a small reduction in J^K_{ms} that is unlike that observed in the aldosterone group (Table

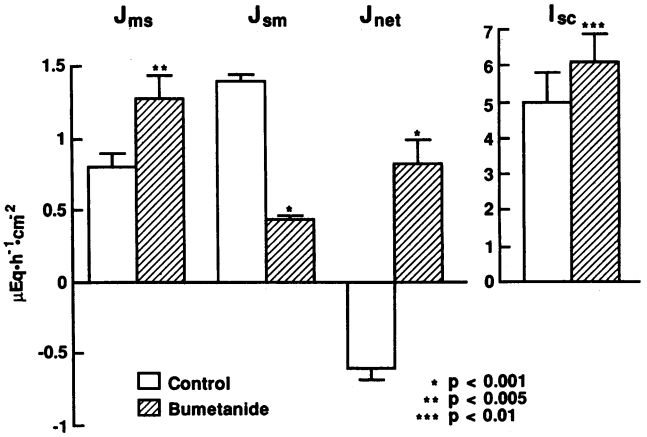

Figure 1. Effect of serosal bumetanide on unidirectional and net potassium fluxes, and short-circuit current in hyperaldosterone animals. Mucosal and serosal sides were bathed with normal Ringer's and bumetanide was added to give a final concentration of 10 μM. The control and post-bumetanide conductance were 8.2 ± 0.4 and 6.3 ± 0.5 $mS \cdot cm^{-2}$ ($P < 0.001$), respectively. Results are from six tissue pairs.

5. Preliminary studies demonstrated that in aldosterone animals the mucosal addition of both 30 and 40 mM TEA and both 5 and 10 mM Ba produced identical changes in J^K_{sm} and that of 10 mM Ba and 30 mM Cs did not alter J^K_{ms} or J^K_{sm}, respectively.

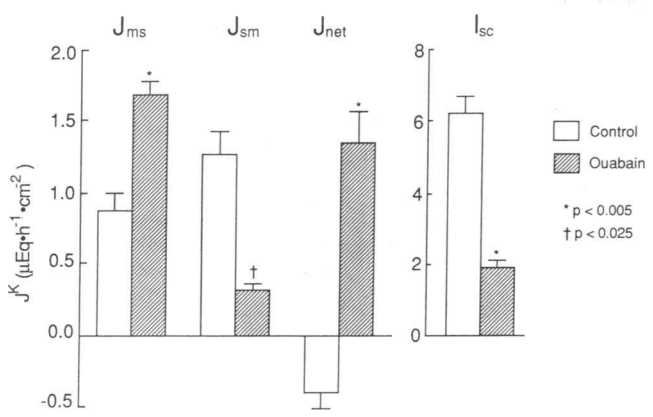

Figure 2. Effect of ouabain on unidirectional and net potassium fluxes and short-circuit current (I_{sc}) in the Na-depleted group. Both sides of the colonic mucosa were bathed with normal Ringer solution and 1 mM ouabain was added to the serosal side. The conductances before and after ouabain were ($mS \cdot cm^{-2}$): 10.3±1.32 vs. 10.7±1.13, P = NS. Results (mean±SE) are from seven tissue pairs.

II), there was no significant change in J^K_{net}. Mucosal Ba also reduced the active component of J^K_{sm} by 100% without an apparent effect on J^K_{ms}. Thus both TEA and Ba totally abolished the active component of J^K_{sm} in normal animals. In contrast, in the aldosterone group the inhibition of J^K_{sm} by TEA was significantly greater than that by Ba (80 and 42%, respectively). Thus, the TEA-insensitive component of J^K_{sm} was significantly smaller than the Ba-insensitive component (0.39±0.06 and 0.92±0.12 $\mu eq \cdot h^{-1} \cdot cm^{-2}$, respectively; $P < 0.005$). This analysis suggests that in normal animals the apical K channel is both TEA- and Ba-sensitive, but that aldosterone induces (or activates) a new population of K channels which exhibit a greater sensitivity to TEA than to Ba.

Relationship between Na absorption and K secretion. A relationship between Na absorption and K secretion has frequently been suggested. However, previous studies in the distal colon of hyperaldosterone animals have demonstrated that amiloride has no effect on K secretion even though electrogenic Na absorption is inhibited (2, 13). It is possible therefore, that K secretion could be sustained by serosal Na uptake alone when apical Na entry is inhibited. In this study when Na was absent from the serosal solution (but present in the mucosal solution), the active component of J^K_{sm} was reduced by ~ 42% with no apparent change in Na absorption as indicated by the I_{sc} (Table I). Under these conditions the addition of mucosal amiloride (10 μM) resulted in a further substantial decrease in J^K_{sm} simultaneous with a further rise in J^K_{ms}, resulted in a large net absorption of K (Fig. 4). At the same time the I_{sc}, which reflects electrogenic Na absorption (2, 13), was reduced from 7.6±0.6 to 1.8±0.1 $\mu eq \cdot h^{-1} \cdot cm^{-2}$, $P < 0.001$. These results suggest that under certain conditions (i.e., when K and Na uptake via the basolateral Na-K-2Cl cotransport system is inhibited) part of the K secretory flux appears linked to Na absorption.

Discussion

The movement of K in the rat distal colon is the result of both active and passive transport processes and both absorptive and secretory processes. In vivo there is net secretion that in large part represents potential dependent K movement (1). In contrast, under short circuit conditions net K absorption is observed that appears to be electroneutral, Na dependent, probably Cl dependent, and consistent with a K-H exchange (2). Active K transport can regulate net K movement in that K depletion augments active K absorption while aldosterone and K loading induce active K secretion (2, 14).

This study was designed to characterize aldosterone-induced electrogenic K secretion in rat distal colon and has demonstrated that this transport process requires (*a*) both serosal Na and serosal Cl; (*b*) both the Na,K-pump and Na-K-2Cl cotransport for K uptake across the basolateral membrane; and (*c*) an aldosterone-induced apical TEA-sensitive, Ba-sensitive K channel for K exit across the apical membrane. A basolateral K channel appears to be absent based on sensitivity to the putative K channel blockers TEA, Ba, and Cs. In the normal colon the small active component of J^K_{sm} could be completely accounted for by an apical TEA- or Ba-sensitive K channel and a basolateral Na-K-2Cl cotransport process.

Table II. Effect of Mucosal TEA, Ba, or Cs on Unidirectional and Net Potassium Fluxes, and on Electrical Parameters in the Distal Colonic Mucosa of the Aldosterone Group

	J_{ms}	J_{sm}	J_{net}	I_{sc}	PD	G	n
			$\mu eq \cdot h^{-1} \cdot cm^{-2}$		mV	$mS \cdot cm^{-2}$	
A. Ringer's	0.67±0.07	1.21±0.05	−0.54±0.11	3.5±0.6	9.3±2.2	10.9±1.1	7
+TEA	1.03±0.11	0.39±0.06	0.63±0.15	4.2±0.4	9.7±1.9	13.0±1.6	
P	<0.005	<0.001	<0.001	<0.025	NS	<0.01	
B. Ringer's	0.79±0.14	1.46±0.05	−0.67±0.11	5.7±0.4	18.2±2.3	8.9±0.9	6
+Ba	0.95±0.14	0.92±0.12	0.03±0.10	5.7±0.3	18.6±2.1	8.6±0.9	
P	NS	<0.005	<0.01	NS	NS	NS	
C. Ringer's	1.08±0.10	1.81±0.13	−0.73±0.20	4.9±0.4	11.6±1.6	13.2±2.4	4
+Cs	1.03±0.16	1.83±0.15	−0.81±0.31	3.9±0.2	8.7±1.7	13.8±2.2	
P	NS	NS	NS	NS	<0.02	NS	

P (paired *t* test) compares the difference between control and experimental values. When agents were added to mucosal or serosal Ringer's, equimolar choline-chloride was added to the opposite compartment. See legend to Table I for definitions and additional information.

Table III. Effects of Serosal TEA, Ba, or Cs on Unidirectional and Net Potassium Fluxes, and on Electrical Parameters in Distal Colonic Mucosa of the Aldosterone Group

	J_{ms}	J_{sm}	J_{net}	I_{sc}	PD	G	n
		$\mu eq \cdot h^{-1} \cdot cm^{-2}$			mV	$mS \cdot cm^{-2}$	
A. Ringer's	0.69±0.05	1.38±0.03	−0.69±0.08	6.9±0.7	18.9±2.6	10.2±0.8	5
+TEA	0.60±0.05	1.33±0.09	−0.73±0.13	7.5±0.6	18.1±2.0	11.6±1.0	
P	NS	NS	NS	NS	NS	NS	
B. Ringer's	0.78±0.08	1.27±0.14	−0.50±0.14	5.9±1.0	15.7±2.4	10.1±1.1	5
+Ba	0.64±0.05	1.15±0.12	−0.50±0.13	7.6±1.1	20.2±2.6	10.9±1.8	
P	<0.05	NS	NS	NS	NS	NS	
C. Ringer's	0.60±0.06	1.54±0.14	−0.94±0.09	5.3±0.8	14.1±2.6	10.3±0.9	5
+Cs	1.19±0.04	0.42±0.04	0.77±0.04	8.0±1.0	22.6±3.7	10.3±1.8	
P	<0.001	<0.005	<0.001	NS	<0.005	NS	
D. Serosal Cl-free	1.13±0.10	0.61±0.01	0.51±0.10	5.9±0.4	31.9±4.3	5.3±0.7	5
+Cs	1.43±0.12	0.42±0.04	1.01±0.14	5.9±0.4	25.4±3.3	6.8±0.9	
P	<0.001	<0.005	<0.001	NS	<0.02	<0.01	

P (paired *t* test) compares the difference between control and experimental values. See legend to Table I for definitions and additional information.

Role of basolateral Na-K-2Cl cotransport and Na,K-pump in K secretion. A Na-K-2Cl cotransport process exists in a variety of cell types and plays a central role in electrolyte and fluid transport in epithelial tissues (see review, reference 15). Two features of this cotransport systems are its specific inhibition by low dose (micromolar range) of the loop diuretic bumetanide and interdependency of ion fluxes. In this study K secretion (J^K_{sm}) was dependent on both serosal Na and Cl (Table I) and was inhibited by serosal bumetanide (Fig. 1). These experiments, therefore, indicate the existence of a Na-K-2Cl cotransport process on the basolateral membrane of the rat distal colon.[6] Moreover, it appears that a major portion of active K secretion is driven by this cotransport process since in the presence of 10 μM bumetanide,[3] or in the absence of serosal Cl, J^K_{sm} was reduced by 83 and 65%, respectively, in aldosterone animals. (In normal animals bumetanide reduced the small J^K_{sm} to a value equivalent to passive transfer.) In a similar study in rabbit descending colon, both basal and adenosine-stimulated J^K_{sm} were abolished in the absence of serosal Cl or Na, or with serosal furosemide, indicating that only K which enters via the basolateral Na-K-2Cl cotransport is available for K secretion (8).

Chronic dietary Na-depletion results in a marked increase in Na,K-ATPase activity due to an increase in pump density in basolateral membrane (5). The increase in K secretion during hyperaldosteronism could therefore be attributed to the increase in the Na,K-ATPase activity in the basolateral membrane (in addition to an increase in apical K conductance discussed below). Thus, serosal ouabain inhibited J^K_{sm} by ~ 90% (Fig. 2), to a value similar to that obtained with serosal bumetanide (Fig. 1), suggesting a relationship between the Na,K-pump and Na-K-2Cl cotransport such that both transport systems are required for K secretion. It is likely that the Na,K-pump maintains the transmembrane Na-gradient, which is necessary for normal operation of the cotransport process. Unlike bumetanide, however, ouabain produced a large decline in the I_{sc}, indicating inhibition of electrogenic Na absorption.[4] This effect has also been observed in rabbit descending colon where ouabain decreased the I_{sc} and transepithelial PD to near zero levels and reduced J^K_{sm} by 65–100% (7,

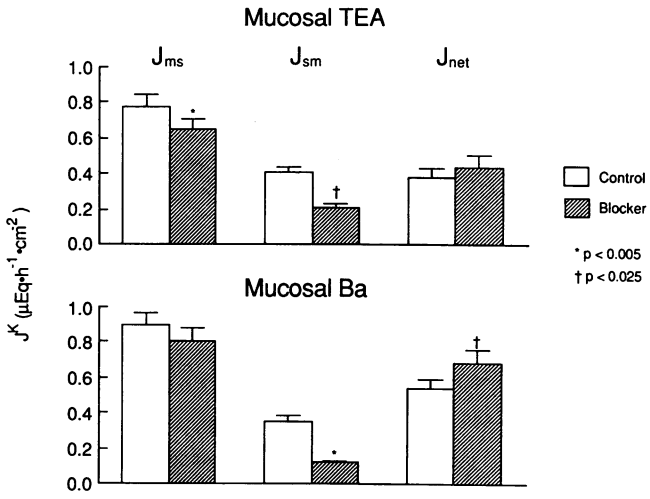

Figure 3. Comparison of the effect of putative K channel blockers on unidirectional and net potassium fluxes across the distal colon of normal animals. The control vs. post blocker I_{sc} ($\mu eq \cdot h^{-1} \cdot cm^{-2}$) were: mucosal TEA, 0.81±0.13 vs. 1.85±0.14, $P < 0.001$; mucosal Ba, 0.51±0.08 vs. 0.80±0.05, $P < 0.005$. Similarly the conductances ($mS \cdot cm^{-2}$) were: 5.6±0.4 vs. 7.5, $P < 0.001$ and 4.8±0.4 vs. 4.5±0.4, P = NS, respectively. Results represent the mean of two 15-min flux periods before and after the addition of 30 mM TEACl or 5 mM $BaSO_4$ to the mucosal bathing solution (and 30 or 5 mM choline-Cl to the serosal bathing solution, respectively). Results are mean±SE from four tissue pairs in each group.

6. Although bumetanide inhibits Na-K-2Cl cotransport as well as KCl cotransport and anion exchange, the $IC_{0.5}$ of bumetanide for Na-K-2Cl cotransport is < 1 μM in flounder intestine (36) and rabbit thick ascending limb of Henle (37). The comparable inhibition (approximately 75%) by 1 mM and 10 μM bumetanide and the 45% inhibition of the active component of J^K_{sm} by 1 μM bumetanide indicates that the $IC_{0.5}$ of bumetanide for K secretion is ~ 1 μM and provides compelling evidence for the presence of Na-K-2Cl cotransport in the rat distal colon.

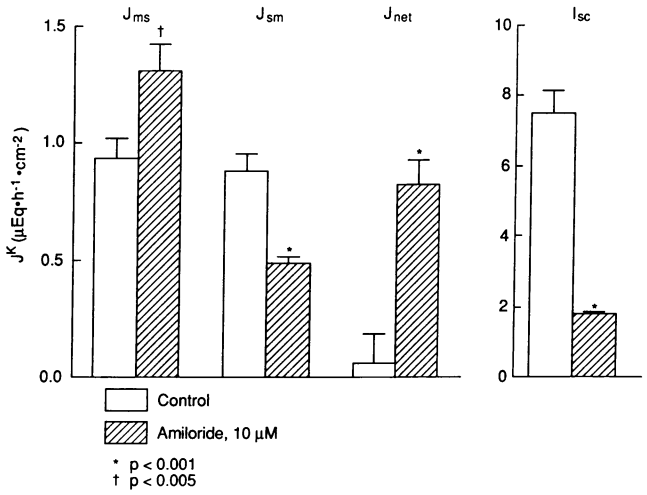

Figure 4. Effect of mucosal amiloride on unidirectional and net potassium fluxes and short-circuit current (I_{sc}) across distal colonic mucosa of dietary Na-depleted animals. The mucosal and serosal sides were bathed with normal and Na-free (choline substituted) Ringer's, respectively. 10 μM amiloride was added. Results are from seven tissue pairs.

16). Neither bumetanide nor removal of Cl from the serosal bathing solution reduced I_{sc} (and thus did not affect the Na,K-pump) but markedly inhibited J^K_{sm} (Fig. 1 and Table I [section B]) suggesting that a major source of K for secretion is that which enters the cell via the Na-K-2Cl cotransport process.

Role of apical and basolateral K channels. Numerous electrophysiological studies have established that K channels, exhibiting variable sensitivity to certain cations, exist in the apical and basolateral membranes of various types of epithelia and that a major function of these channels appears to be the regulation of transepithelial K movement (see review, reference 17). Studies in rabbit cortical collecting duct and distal colon demonstrate an apical membrane conductive pathway for K exit which is blocked by either TEA or Ba (7, 8, 16, 18–20). In the rat distal colon, conventional microelectrode studies in our laboratory have indicated that a TEA-inhibitable K conductance is present on the mucosal side during chronic dietary potassium loading (4). By using these putative K channel blockers the present study provides evidence to suggest that K exit across the apical membrane occurs via K channels since in the aldosterone rat mucosal TEA or Ba, but not Cs, prevented net K secretion (Table II). Although both mucosal TEA and Ba completely inhibited active J^K_{sm} in normal animals (Fig. 3), their effect in the aldosterone group was strikingly different.[5] TEA in Na-depleted animals resulted a substantial (80%) reduction in the active component of J^K_{sm}, but Ba reduced this component by only 42%. These data can best be interpreted to indicate that aldosterone induces (or activates) a new population of K channels which exhibit a higher sensitivity to TEA than to Ba, whereas in normal animals the apical K channel is TEA and Ba sensitive. Similar findings were recently reported (21) in the guinea-pig distal colon where application of TEA or Ba to the mucosal side caused a reversible increase in I_{sc} (an indicator of electrogenic K secretion).

Basolateral K conductance, which is largely Ba inhibitable, is present in a variety of epithelia (17, 18, 21, 22, 23). In this study serosal TEA or Ba had no significant effect on K fluxes in either direction across the distal colon (Table III) indicating the absence of a basolateral TEA- or Ba-sensitive K channels. Note, however, that Gregor has recently suggested that K movement across the basolateral membrane of some epithelial cells (e.g. rabbit cortical thick ascending limb of Henle, Necturus gallbladder) occurs via a KCl cotransport process (24).

Although serosal Cs markedly decreased K secretion, this effect is not primarily a direct effect of Cs on a basolateral K channel for the following reasons: first, in the absence of serosal Cl when Na-K-2Cl cotransport is inhibited, the effect of Cs on J^K_{sm} was small (and may be due to interaction with the Na,K-pump; see reference 25) compared with that in normal Ringer's (Table III [section C and D]). Second, if Cs blocks a basolateral K channel, then its addition to the serosal side should reduce the activity of the Na,K-pump by virtue of decreasing K cycling, which should result in a reduction in electrogenic Na absorption in the aldosterone animal. Such effects have been observed in Necturus urinary bladder, where addition of serosal Ba reduced basolateral K conductance and blocked apical Na channels as reflected in the increase in basolateral and apical membrane resistance, respectively (26). Our results demonstrate that the elevated I_{sc}, an indicator of electrogenic Na absorption, was not reduced in the presence of serosal Cs (Table III [section C]). Together, these findings argue against a Cs-sensitive basolateral K channel and indicate that Cs inhibits Na-K-2Cl cotransport. Of interest is evidence in rectal gland plasma membrane vesicles that Cs interacts with the Na-K-2Cl symporter without being transported (27).

Relationship of K secretion to Na absorption. Traditionally the Koefoed-Johnsen and Ussing model for active Na absorption implies that K uptake across the basolateral membrane is linked to the simultaneous absorption of Na, as would be expected from the coupling between Na efflux and K influx, and mediated by the basolateral membrane Na,K-ATPase (see reference 10). If such a relationship exits, amiloride, which inhibits electrogenic Na absorption, should inhibit K secretion. Studies that have examined this relationship have not always observed such inhibition of K secretion by amiloride. In general, experiments performed under open circuit conditions have shown an inhibition of K secretion (simultaneous with inhibition of Na absorption), e.g. in rabbit cortical collecting tubule (28, 29), whereas those performed under short circuit conditions have not, e.g. in rabbit colon (7, 8, 30). It is likely that the inhibition of K secretion by amiloride under open circuit conditions is due to a decrease in potential-dependent K secretion and not to an effect on active K secretion. In distal colon of Na-depleted rats amiloride-sensitive Na absorption is the predominant Na transport process (2, 6), but in Ringer's solution, under short circuit conditions, amiloride has not inhibited active K secretion (2). In contrast, in the absence of serosal Na in aldosterone animals mucosal amiloride resulted in a prompt reduction in both the I_{sc} and J^K_{sm} (Fig. 4). Since a close coupling between Na absorption and K secretion is only observed (Fig. 4) when the basolateral Na-K-2Cl cotransport system is inhibited, we interpret these results to indicate that the major fraction of K secretion is derived from Na-K-2Cl cotransport which is not linked to Na absorption. These results also suggest that a portion of K secretion occurs in Na absorbing cells.

Relation between K secretion and K absorption. Alteration in any of the processes by which K enters the cell, namely

inhibition of basolateral Na,K-pump or Na-K-2Cl cotransport process, resulted in a marked stimulation of J^K_{ms} in the aldosterone animal (Tables I and II; Figs. 1, 2, and 4). These results suggest that a subpopulation of colonocytes might contain both the K absorptive and K secretory processes because there was significant correlation ($P < 0.02$) between inhibition of J^K_{sm} and augmentation of J^K_{ms} (Fig. 5). In the absence of more definitive information we would speculate that these two transport processes may also reside in separate cells.

The mechanism responsible for this coupling between bidirectional transepithelial K movement is unknown but a reasonable explanation could be advanced based on our results and the arguments presented previously by Wills and Biagi (31). In rabbit descending colon (31, 32) and in rabbit (33) and Necturus (34) gallbladders intracellular K activity is normally above its electrochemical equilibrium and within the range of 73–86 mM. This would provide the gradient for conductive K exit across the apical membrane. Indeed, in guinea pig distal colon, high K concentration (105.4 mM) in the mucosal bath abolished the I_{sc}, and therefore, K secretion (21). As a result, it is reasonable to suggest that the following speculation may explain the relationship between the decrease in J^K_{sm} and the increase in J^K_{ms}. A decrease in uptake of K across the basolateral membrane most likely decreases intracellular K activity and thereby reduces the potential across the apical membrane. The resulting decrease in apical membrane K conductance would lead to a decrease in apical membrane K recycling and thus an increase in J^K_{ms}. It is most likely that the increase in J^K_{ms} occurs in aldosterone but not in normal animals since there is a three-fold increase in J_{max} without a change in Km of the active K absorptive process in aldosterone compared to normal cells (35). However, the available data do not permit a definitive conclusion whether the K absorptive and secretory processes are only located in one cell type or are also present in different epithelial cells.

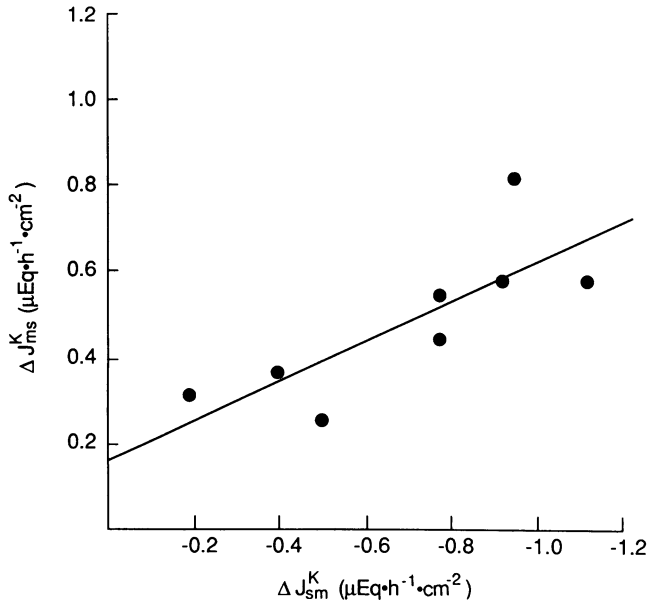

Figure 5. Relationship between inhibition of serosal-to-mucosal (J^K_{sm}) and stimulation of mucosal-to-serosal (J^K_{ms}) K movement after alteration of basolateral K uptake in the aldosterone group. ΔJ^K_{ms} and ΔJ^K_{sm} represent the difference between control values and those measured after inhibition of basolateral K uptake (see Tables I [section B] and III [section B], and Figs. 1, 2, and 4). A line was fitted (by least-square analysis) to the data points: ordinate-intercept = 0.16±0.11±, slope = 0.46±0.15; $r = 0.79$ ($P < 0.02$).

In summary, these findings demonstrate that in the normal rat distal colon, in which there is net K absorption in vitro under short-circuit conditions, the small J^K_{sm} could be completely accounted for by an apical TEA- or Ba-sensitive K channel and a basolateral Na-K-2Cl cotransport process. In the aldosterone animal, net K secretion is associated with the stimulation (or induction) of K channels in the apical membrane that are sensitive to both TEA and Ba and requires Na,K-ATPase and Na-K-2Cl cotransport for K entry across the basolateral membrane.

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