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K^+ Channels and Colonic Function

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1 Introduction

K^+ channels are required both, for absorption and secretion of fluids and electrolytes in epithelia. Recent findings in molecular biology and electrophysiology have given new insight into the nature of colonic K^+ channels. Their properties and their putative molecular identity will be described in detail. The crucial role of their regulation for the net transport of Na^+, K^+, H_2O and Cl^- will be reviewed in this chapter. In addition the role of K^+ channels in the cytosolic homeostasis of the single enterocyte i.e. regulation of cell volume, control of transport, and differentiation will be discussed.

Although there are distinct differences between species in colonic function and anatomy the underlying mechanisms for absorption or secretion are similar. The activity of transport proteins, however, depends on the needs for salt and water balance of the respective species and results in different functional phenotypes. In this review we therefore do not diverge to species differences but emphasise the integration of experimental findings to arrive at the basic mechanisms.

K^+ channels are a prerequisite for epithelial transport in the large intestine. Their function determines weight and electrolyte composition of the stool. A volume of about 1500 ml/day enters the colon, less than 200 g are excreted per day. In addition the colon is the site of action for laxatives as well as for bacterial toxins. The composition of stool is given in Table 1 [30,99,244,285].

Nutrients like glucose and amino acids are absorbed already in the small intestine and there is no evidence for glucose or amino acid absorption in the colon of adults. Short chain fatty acids (SCFA) and gasses like H_2, CO_2, CH_4, and H_2S are produced in the large intestine by bacterial fermentation of undigested carbohydrates and proteins. SCFA form the major part of anions in the colon lumen. K^+, Na^+ and NH_4^+ are the respective cations.

The major task of the colon under physiological conditions is the absorption of Na^+, SCFA, HCO_3^-, Cl^-, and H_2O as well as the secretion of K^+ and mucus. Besides secretion of K^+ and mucus the secretory function of the colon becomes only evident when physiological or pathophysiological stimuli act on enterocytes. Under these conditions the vectorial transport of Na^+, H_2O and Cl^- is reversed and stool volume can increase up to 6-fold and more.

Some congenital diseases linked to defects of distinct membrane transport proteins elucidate the understanding of transport mechanisms and

Table 1. Composition of stool

	Concentration	Excretion/day
H_2O		90 ml
Solids		21 g
pH	5.9–8.5	
Bilirubin		5–20 mg
Urobilinogen		57–200 mg
Bile acids	9 mmol/l	200–800 mg
Lipids		0.1–11 g
Glucose, Amino acids	<1 mmol/l	
Cations		
Na^+	5–125 mmol/l	0.5–12.5 mmol
K^+	30–200 mmol/l	3–20 mmol
Ca^{2+}		8–33 mmol
Mg^{2+}		5–15 mmol
NH_4^+	14–20 mmol/l	
Anions		
Cl^-	5–30 mmol/l	0.5–3 mmol
HCO_3^-	< 30 mmol/l	<3 mmol
Phosphate		10–25 mmol
Organic anions (SCFA)	100–400 mmol/l	10–40 mmol

facilitate the ranking of functional relevance of the respective pathways. Examples of disorders which cause a colonic phenotype are cystic fibrosis with a defective luminal Cl^- conductance [151], congenital chloride diarrhoea with a functional defect of the luminal $Cl^-/HCO3^-$ exchanger [119,137] and congenital sodium secretory diarrhoea with a functional defect of the luminal Na^+/H^+ exchanger [82].

Electrolyte transport in the mammalian colon has been reviewed in detail [12,120,223]. A short summary and update of the basic mechanisms will be given in the following section.

2
Anatomy and Function of the Colon

Cecum, proximal and distal colon differ in their functional properties and embryological origin. Cecum and proximal colon develop from the midgut. The distal colon develops from the hindgut. Cecum and proximal colon are intermediate conductance epithelia with a transepithelial resistance of about 100 Ωcm^2. The distal colon is moderately tight with a fourfold higher resis-

tance [35]. Except for the distal colon the information about carrier proteins and ion channels which are involved in electrolyte transport in these segments is limited, and no information is available on the single channel level from the intact tissue. SCFA are absorbed in the undissociated form by nonionic diffusion [31] and to a larger portion as organic anions. They enter the epithelium probably coupled to HCO_3^- secretion [270] and with Na^+/H^+ exchange working in parallel [236]. There is also evidence for several other transport mechanisms for SCFA, however, their functional relevance is still undefined [192,218].

2.1 Cecum

In the cecum Na^+ absorption takes place in electrogenic and to a small extend in electroneutral fashion. The mechanism responsible for the considerable amount of electrogenic Na^+ absorption in this colon segment is not clearly defined. It has been suggested that Na^+- or cation-selective channels with pharmacological properties different from the epithelial Na^+ channel ENaC (no inhibition by amiloride) are present in the luminal membrane [238–240]. Electroneutral Na^+ absorption is probably coupled to the action of Na^+/H^+ and $Cl^-/HCO3^-$ exchange in the luminal membrane. There is also evidence for electrogenic HCO_3^- secretion [115]. For K^+ secretion in the cecum K^+Cl^- cotransport has been proposed [78].

2.2 Proximal Colon

In the proximal part of the colon Na^+ is absorbed mainly electroneutrally. Luminal Na^+/H^+ exchange is the predominant Na^+ uptake mechanism. It works in parallel with luminal $Cl^-/HCO3^-$ exchange. In addition a combined action of luminal Na^+/H^+ exchange with basolateral $Na^+(HCO_3^-)_{(x)}$ export has been proposed [83,237]. Although conductive Na^+ absorption has been described, aldosterone increases only electroneutral Na^+ absorption [66,83]. Another agonist that stimulates electroneutral Na^+ absorption in the proximal colon is epinephrine which acts in these epithelial cells via α_2 adrenergic receptors [235]. There is evidence for luminal and basolateral K^+ conductances which are both stimulated via carbachol with cytosolic Ca^{2+} as the second messenger [231]. Like in the distal colon the total basolateral K^+ conductance is decreased after cAMP stimulation [231].

2.3 Distal Colon

In the distal colon Na^+ absorption occurs through electroneutral and electrogenic pathways [233]. Figure 1 shows the respective cell models with the cellular transport mechanisms. Electrogenic absorption of Na^+ occurs via ENaC [27,160] which is located in the luminal membrane of crypt surface cells. Amiloride directly inhibits this component of Na^+ absorption at micromolar concentrations. The amiloride-sensitive Na^+ conductance in the distal colon is under control of mineralocorticoids which increase this conductance mostly by post-transcriptional regulation of ENaC via aldosterone-induced proteins (AIPs) [269].

Another pathway for Na^+ absorption is the electroneutral Na^+/H^+ exchanger [212]. Na^+/H^+ exchange occurs in parallel with Cl^-/HCO_3^- exchange [213] resulting in NaCl uptake. There is evidence for the expression of several NHE isoforms in colon mucosa [17,173,200,272], NHE1 works as a

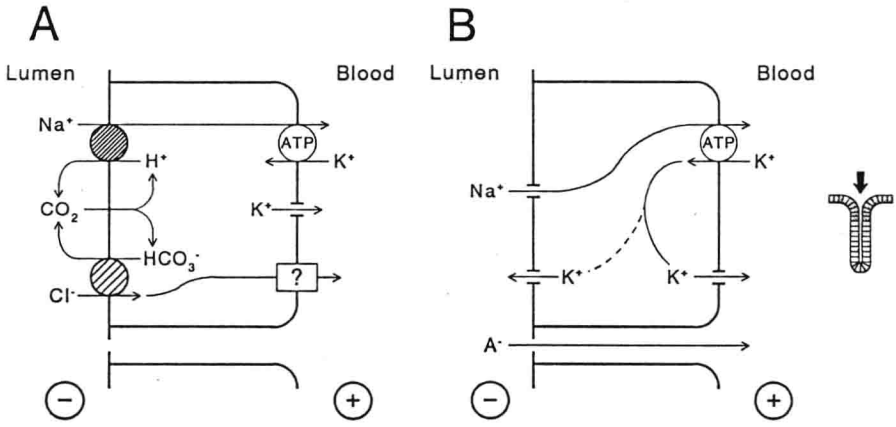

Fig. 1. Cell models for the mechanisms of transcellular Na^+ absorption predominantly located in colon crypt surface cells. **A** Electroneutral Na^+ absorption: Na^+ enters the cell via Na^+/H^+ exchange in the luminal membrane. Parallel secretion of HCO_3^- into the lumen by Cl^-/HCO_3^- exchange. Formation of CO_2 in the lumen and recycling into the cell. On the basolateral side Na^+ is extruded by Na^++K^+-ATPase. The mechanism of Cl^- exit is not known at this stage. **B** Electrogenic Na^+ absorption: Na^+ enters the cell via epithelial Na^+ channels (ENaC). On the basolateral side Na^+ is extruded by Na^++K^+-ATPase. K^+ recycles via basolateral K^+ channels or leaves the cell through luminal K^+ channels. Luminal Na^+ and K^+ channels are regulated by mineralocorticoids and K^+ metabolism. The lumen-negative transepithelial voltage provides the driving force for paracellular anion absorption

housekeeper engaged in pH homeostasis and is located in the basolateral membrane but it is not involved in Na^+ absorption in the colon. The precise localisation and function of NHE2 and NHE3 are still discussed but both isoforms are the likely candidates to maintain electroneutral colonic Na^+ absorption [67,121,184]. Moreover there is low NHE4 expression in the colon. In addition a Cl^--dependent form of Na^+/H^+ exchanger was postulated for crypt base cells [214]. A variety of agonists act on distal colon function either directly via the epithelial cells or indirectly via enteric neurones or endocrine cells. They are located in the lamina propria or in the crypt itself. A summary of these factors and their effects is given in Table 2.

Electrogenic and electroneutral Na^+ absorption are controlled in opposite direction by aldosterone and glucocorticoids. Elevation of aldosterone enhances electrogenic Na^+ absorption and inhibits electroneutral Na^+ transport. Glucocorticoids favour electroneutral transport at low concentrations and mimic aldosterone action at high concentrations [9,234,265]. The different proportions of electrogenic and electroneutral Na^+ transport between species can, in part, be explained by varying aldosterone concentrations. They depend on the dietary intake of Na^+ and K^+, i.e. a relatively high Na^+ intake in rats and man, and a vegetarian low Na^+ diet in rabbits.

As mentioned above the distal colon epithelium is moderately tight (ca. 200–400 Ωcm^2) [35,92] and allows the generation of remarkable transepithelial potential differences of up to -100 mV. Along this transepithelial electrical driving force paracellular ion transport can occur against existing concentration gradients.

Secretion mainly occurs in the crypt middle and base. In these cells Cl^- is secreted transcellularly involving basolateral uptake via the loop-diuretic-sensitive $Na^+2Cl^-K^+$ cotransporter [70,117] and luminal exit of Cl^- via Cl^- channels. Opening of luminal Cl^- channels depolarises the luminal membrane and thereby creates a lumen-negative transepithelial voltage. K^+ mainly leaves the cell via basolateral K^+ channels and thus hyperpolarises the basolateral membrane providing the driving-force for luminal Cl^- exit. Cations follow paracellularly driven by the transepithelial voltage. Basolateral K^+ recycling is necessary for any electrogenic Cl^- secretion in the colonic mucosa. Inhibition of the respective K^+ channels blocks electrogenic Cl^- secretion completely [15,166,280]. The opening of luminal K^+ channels in the presence of a luminal Cl^- conductance causes electroneutral (i.e. parallel) secretion of K^+ and Cl^-. Figure 2 shows a cell model for electrolyte secretion in the distal colon.

Until now there is only limited information about the properties of the paracellular pathways in epithelia [92,247] although there has been sub-

Table 2. Agonists acting on colonic function

Agonist	Origin	Target	Receptor	Messenger	Effect	References
Amines						
Acetylcholine	N	E	M_3	IP_3, Ca^{2+}	secretion	[59,127,136,199]
		ET (SM)	M_1	NO	relaxation	
		N	nicotinic		secretion	
Serotonin	M, EC	E	5-$HT_{(x)}$	cAMP	secretion	[98,222]
		SM			contraction	
		N	VIP		secretion	
Noradrenaline/	N	SM	α1	Ca^{2+}	contraction	[45,122,136,246,253]
Adrenaline		N	α2			
		E(s)	α2	cAMP↓, Ca^{2}	absorption,	
		E(s)	β1	cAMP	K^+ secretion	
		N	β2			
Histamine	M	E	H_1	Ca^{2+}	secretion	[181,276]
		N		PGE_2	secretion	
Purines						
ATP	N	E	P_{2Y}	Ca^{2+}	secretion	[98,156]
		SM	P_{2X}	Ca^{2+}	contraction	
Adenosine	M	E			secretion	[18]
Peptides						
VIP	N	E		cAMP	secretion	[23,98,136]
		N/SM			relaxation	
GRP (gastrin-releasing peptide)		N			secretion	[79,261,262]

Tachykinins (substance P, neurokinin A and B, neuropeptide B)	N	E N		cAMP	secretion variable	[40,98,136,154]
NPY (neuropeptide Y), PYY (peptide YY)	N	E	Y_1	cAMP↓ NA(α2)	absorption relaxation	[19,136,249]
Somatostatin	EE N	E N	SSTR1,2,5		absorption secretion ↓	[55,77,136,253,277]
Opioids		N			absorption	[136,253]
Endothelins		E, N	ET1R		secretion	[150]
Guanylin/uroguanylin		E	GC-C	cGMP	secretion	[139]
Eicosanoids						
PGE_2	M	E, N	EP4	cAMP	secretion	[54,63,187]
$PGF_{2\alpha}$	M	N			secretion	[85]
PGI_2	M	N			variable	[85]
PGD_2		N			secretion ↓	[60,94]
HETE, HPETE	E, SubE				secretion	[10,41]
Gas						
NO (Nitric oxide)	N, M, L	N	PGE_2, cGMP		secretion	[98,287]
Bacterial toxins						
Cholera toxin		E N (E, SM)	GM_1	cAMP 5-HT, VIP	secretion secretion, relaxation	[98,207,295]
Toxin of *Y. enterocolitica*		E		cGMP	secretion	[125]
Heat-stable *E. coli* toxin		E SubE		cGMP PGE_2	secretion	[198,207]

Table 2. (continued)

Agonist	Origin	Target	Receptor	Messenger	Effect	References
Heat labile *E. coli* toxin		E		cAMP	secretion	[295]
Toxin A of *C. difficile*		M			secretion, inflammation	[98]
Cytokines						
Tumour necrosis factor α	L				secretion, inflammation	[229]
Laxatives						
Ricinoleic acid		SM			contraction	[98]
Anthraquinones				PGE2, Histamine, 5-HT, NO	secretion	[128]
Bile salts		E, N, SubE			secretion	[76]

Abbreviations: N – neurones; E – enterocytes; M – mast cells; EC – enterochromaffin cells; EE – enteroendocrine cells; SubE – subepithelial cells; ET – endothelial cells; SM – smooth muscle cells; L – leukocytes; (s) species differences.

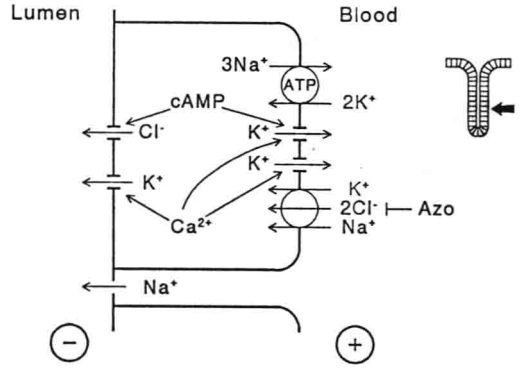

Fig. 2. Cell model for the mechanism of transcellular Cl^- secretion predominantly located in crypt mid and base cells. Cl^- leaves the cell via cAMP-activated Cl^- channels (CFTR) in the luminal membrane. In parallel K^+ leaves the cell via (mainly basolateral) K^+ channels providing the driving force for luminal Cl^- exit. These K^+ channels are differentially regulated by cAMP and Ca^{2+}. The transepithelial voltage drives paracellular cation secretion. The activation of a luminal K^+ conductance under the influence of mineralocorticoids, K^+ metabolism and agonists acting via $[Ca^{2+}]_i$ determines transepithelial voltage and KCl secretion. Uptake of Cl^- occurs via $Na^+2Cl^-K^+$ cotransport in the basolateral membrane which can be inhibited by the loop diuretic azosemide (Azo)

stantial progress in the analysis of the structure and architecture of tight junctions [185]. Targeting, assembly, and phosphorylation of the respective proteins and their interaction with the cytoskeleton might be involved in colonic tight junction regulation. In the functional state of Na^+ absorption the shunt pathway should be selective for anions, whilst during secretion a cation selectivity is required. This problem could be solved by different properties of tight junctions along the crypt axis. Another possibility would be the differential regulation of colon crypt tight junctions depending on stimulation of absorption or secretion. In the ileum there is evidence for cAMP-dependent regulation of the tight junction permeability [11]. Whether the selectivity properties of colonic crypt tight junctions are regulated and whether they differ along the crypt axis requires further investigation. Refined techniques to examine this issue are now available [92,93,293].

2.4 Crypt Axis and Function

The colonic mucosa is formed by colonic crypts. The colonic crypt represents the smallest functional unit of colonic mucosa and can be divided into crypt surface, crypt middle, and crypt base. This division has a functional