Salt and water absorption in the human colon: a modern appraisal

Introduction
The past 20 years have seen many advances in all aspects of colonic physiology, and the unrelenting appearance of new information is daunting to clinicians and scientists alike. Nevertheless, we should not lose sight of the fact that the main function of the human colon is to absorb about 90% of the 1.5–2 litres of ileal effluent which passes daily through the ileocaecal valve. In mammalian species, the key determinant of colonic water absorption is the rate of Na+ absorption. We now know that Na+ transport processes are not distributed uniformly throughout the human colon, a concept which has important clinical implications. This review provides an update on the basic mechanisms underlying salt and water transport in the human colon in health and disease, and highlights several interesting areas for future research.

General description of Na+ absorptive processes
The human colon has a nominal mucosal surface area of about 2000 cm², but in reality the total absorptive area is even greater because colonic crypt cells are capable of absorption as well as secretion. Although it is well established that the rates of colonic salt (Na+ plus Cl−) and water absorption are directly related, it is only recently that we have begun to appreciate the array of Na+ absorptive processes present in human colon. These show considerable intrinsic segmental heterogeneity. This explains, at least in part, why the colon’s capacity for sodium and water absorption in vivo is greater in the proximal (ascending) segment than in the distal (descending and sigmoid colon/rectum) segment. Several different active (transcellular) Na+ absorptive processes exist in human colon. It will become clear that segmental differences in the distribution and regulation of these processes play an important role in colonic Na+ salvage during periods of salt deprivation, in the presence of mucosal inflammation, and after surgical resection.

Electrogenic Na+ Absorption
Electrogenic Na+ absorption is present throughout the human colon. The hallmark of this process is the presence of Na+ channels located predominantly in the apical membrane of surface colonocytes, through which Na+ ions diffuse into the cell along a favourable electrochemical gradient. This gradient reflects the low intracellular Na+ concentration (<15 mM) and the negative intracellular electrical potential difference. Active extrusion of Na+ ions across the basolateral membrane is mediated by the ouabain sensitive electrical Na+ pump (Na+,K+-ATPase). Each pump cycle results in the extrusion of three Na+ ions in exchange for the basolateral uptake of two K+ ions, resulting in the net transfer of one positively charged (Na+) ion across the basolateral membrane (fig 1). As the potential difference across the basolateral membrane (negatively charged with respect to the serosal surface) exceeds that across the apical membrane (negatively charged with respect to the luminal surface), a substantial lumen negative transmucosal potential difference (25–45 mV) is normally present in healthy human colon in vivo and in vitro, which largely reflects electrogenic Na+ transport. Inhibition of electrogenic Na+ absorption, and a reduction or abolition of the transepithelial potential difference. In the human colon, however, the nature and distribution of apical Na+ conductances, and their responses to amiloride, are more complex. Thus, under in vitro conditions, addition of 0.1–1.0 mM amiloride to distal colon decreases the potential difference by 61–94% and the short-circuit current (an indicator of net transepithelial ionic flow when the potential difference is electrically “clamped” to zero) by 76–93%, whereas in proximal colon the electrical changes are minimal. Furthermore, 1 µM aldosterone stimulates the amiloride sensitive short-circuit current in isolated human distal colon after five hours, but has no effect in human proximal colon despite the presence of aldosterone receptors in this segment. The speed of action of aldosterone in human distal colon is consistent with receptor mediated induction of one or more of the three Na+ channel subunits (designated α, β and γ, see below) or an additional channel regulatory protein, the activation of “latent” apical Na+ channels, or a combination of these possibilities.

Recent progress in defining the structure–function relations of epithelial Na+ channels is likely to provide new insights into the nature and regulation of Na+ channels in human colon. The primary structure of the aldosterone induced, amiloride sensitive Na+ channel in rat distal colonic epithelium has been established by expression cloning. Coexpression studies have shown that all three homologous subunits (designated α-, β- and γ-ENaC) are required to produce maximal amiloride sensitive Na+...
ELECTRONEUTRAL NaCl ABSORPTION
Studies in isolated sheets of human sigmoid colon and rectal mucosa have shown that 1 mM amiloride applied apically, decreases the short-circuit current to a far greater extent than net Na⁺ absorption, which suggests that a substantial fraction of net Na⁺ absorption is mediated by a process (or processes) other than amiloride sensitive electrogenic Na⁺ transport between different regions of the human colon.

Although electroneutral NaCl absorption has been studied most extensively in rat distal colon, there is good reason to believe that the key components of this process are also present in human colon. In rat distal colon, basal net Na⁺ absorption is electroneutral, Cl⁻ dependent, and inhibited by 1 mM amiloride (a concentration which inhibits apical Na⁺–H⁺ exchange). Furthermore, net Cl⁻ absorption and net Na⁺ absorption are equal and probably regulated by intracellular pH, as both are inhibited by acetazolamide, a carbonic anhydrase inhibitor that reduces endogenous HCO₃⁻ production. Thus, it is now generally accepted that electroneutral NaCl absorption in rat distal colon reflects dual Na⁺–H⁺:Cl⁻–HCO₃⁻ exchanges operating in parallel in the apical membrane.

Studies performed in human colon have been fewer and perhaps less stringent than those performed in rat distal colon. Nevertheless, removal of Na⁺ from human proximal and distal colon in vitro decreases the unidirectional Cl⁻ flux from mucosa to serosa and abolishes net Cl⁻ absorption, a response consistent with apical Na⁺ coupled Cl⁻ uptake. Theophylline, a cAMP mediated Cl⁻ secretagogue which stimulates net Cl⁻ secretion in other intestinal epithelia by inhibiting apical Na⁺ coupled Cl⁻ uptake, has a different effect in the human transverse and distal colon, where it stimulates electrogenic Cl⁻ secretion, a process which involves the activation of apical Cl⁻ channels. Other in vitro studies have shown that, to a degree, active Cl⁻ absorption in human distal colon reflects an electroneutral, Na⁺ independent process consistent with Cl⁻–HCO₃⁻ exchange.

Indeed, in vivo perfusion studies indicate that roughly 25% of the Cl⁻ absorbed by human colon reflects Cl⁻–HCO₃⁻ exchange, the remainder reflecting passive Cl⁻ transport along the favourable electrical gradient (lumen negative potential difference) generated by electrogenic Na⁺ absorption. Taken together, these observations suggest that electroneutral NaCl absorption throughout the human colon (apart from the caecum) reflects dual apical Na⁺–H⁺:Cl⁻–HCO₃⁻ exchanges (fig 2), although the presence of a simpler Na⁺ coupled Cl⁻ uptake process cannot be excluded.
SHORT CHAIN FATTY ACID COUPLED Na⁺ ABSORPTION

Recent studies have highlighted the role of short chain fatty acid (SCFA) coupled Na⁺ absorption in the colonic salvage of carbohydrate, Na⁺ and water. Human caecum and proximal colon have high luminal concentrations of organic nutrients (non-starch polysaccharides from plant cell walls, and proteins not absorbed by the small intestine) which maintain high bacterial growth rates. Against this fermentative background, antiperistalsis ensures retention and thorough mixing of faeces in the proximal colon, which is the site of maximal SCFA production. SCFA absorption is concentration dependent and occurs most readily in the proximal colon, which is the prime site for both energy conservation and SCFA dependent Na⁺ and water absorption. Nutrient concentrations, bacterial growth rates and fermentation rates decrease steadily moving in a caudal direction, and there is a 30% fall in total SCFA concentration and a progressive rise in luminal pH in the distal colon compared with the proximal colon. Of the three SCFAs (acetate, propionate and butyrate) present within the colonic lumen, butyrate is the most important physiologically despite accounting for only about 20% of the total in molar terms. Butyrate serves as a major source of energy for human colonocytes and plays a crucial role in colonoocyte growth and differentiation.

Although it has been clear for some time that SCFAs enhance Na⁺, Cl⁻ and water absorption in human colon, details of the underlying mechanisms have had to await studies in rat distal colon isolated under voltage clamp conditions. Thus, under HCO₃⁻-free conditions, 25 mM mucosal butyrate produces a twofold increase in both Na⁺ absorption and Cl⁻ absorption without changing short-circuit current, in keeping with stimulation of electroneutral NaCl absorption. Mucosal addition of 1 mM amiloride inhibits both butyrate stimulated Na⁺ and Cl⁻ absorption, and Cl⁻ removal from the bathing solution inhibits butyrate stimulated Na⁺ absorption. These observations suggest that Na⁺–H⁺ and Cl⁻–butyrate exchanges operate in parallel at the apical membrane. Furthermore, the Cl⁻–butyrate exchange and the Cl⁻–HCO₃⁻ exchange seem to be two entirely distinct apical anion transport mechanisms. From these experimental findings arose the initial model linking butyrate absorption to electroneutral NaCl absorption, which entailed protonated butyrate moving across the apical membrane by non-ionic diffusion (fig 3). One problem associated with this model is that the pK of SCFA (4.2–4.8) is considerably lower than the luminal pH (7.0–7.4), so that <1% of luminal SCFAs is protonated. This obviously runs counter to the idea that non-ionic diffusion is the dominant SCFA absorptive mechanism.

Recent studies using apical membrane vesicles (AMV) prepared from rat distal colon and human proximal and distal colon have provided additional insights into the apical butyrate uptake mechanism and its relation to butyrate stimulated electroneutral NaCl absorption. Firstly, non-ionic diffusion is an insignificant component of total butyrate uptake, and is probably restricted to paracellular pathways. Secondly, an outwardly directed HCO₃⁻ gradient is an absolute requirement for butyrate uptake, which is consistent with the notion that butyrate–HCO₃⁻ exchange is the dominant apical butyrate uptake mechanism. Thirdly, butyrate stimulated Cl⁻ absorption reflects recycling of intracellular butyrate via an apical Cl⁻–butyrate exchange. In addition, butyrate stimulated electroneutral NaCl absorption is negligible in the distal colon of aldosterone treated rats, in which the mineralocorticoid abolishes apical Na⁺–H⁺ exchange while simultaneously inducing apical Na⁺ channels, providing strong evidence that functional Na⁺–H⁺ exchange is critical for SCFA stimulated electroneutral NaCl absorption. Taken together, these observations support the currently proposed model of butyrate enhanced electroneutral NaCl absorption (fig 4). The main features of this model are: predominantly transcellular butyrate absorption, involving apical butyrate uptake mediated via butyrate–HCO₃⁻ exchange, leading to intracellular acidification and activation of apical Na⁺–H⁺ exchange; partial recycling of intracellular butyrate to the lumen via an apical Cl⁻–butyrate exchange; and a relatively small component of butyrate absorption by paracellular non-ionic diffusion.

NA⁺ AND WATER ABSORPTION BY COLONIC CRYPTS

The idea that (Na⁺) absorptive processes are restricted to surface colonocytes and small intestinal villous cells, whereas (Cl⁻) secretory processes are restricted to colonic and small intestinal crypt cells, is convenient and has been generally accepted for more than 20 years. This spatial distribution model of intestinal electrolyte transport evolved from studies in mammalian small intestinal and colonic epithelia using a variety of experimental approaches. However, the results of studies using microelectrode and voltage scanning techniques challenged the idea of a clear demarcation between the sites of electrolyte absorption and secretion in intestinal epithelia. Thus, both crypt and surface/villous cells were found to secrete Cl⁻ and water when stimulated by cAMP or cAMP mediated secretagogues.

The next milestone in the evolution of our view about the distribution of absorptive processes, at least in the colon, was the suggestion by Naftalin et al that crypt cells

\[ H^+ \leftrightarrow HCO_3^- \]

\[ HBut \leftrightarrow H^+ + But^- \]

\[ Na^+ + HCO_3^- \leftrightarrow But^- + H^+ \]

\[ Na^+ + Cl^- \leftrightarrow But^- + H^+ \]
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INFLAMMATORY BOWEL DISEASE

Decreases in net Na⁺ and Cl⁻ absorption, resulting in impaired water absorption or water secretion, are the main electrolyte transport abnormalities in ulcerative colitis and Crohn’s disease of the colon. Despite recent studies showing that soluble inflammatory mediators released from inflamed human colonic mucosa elicit electrogenic Cl⁻ secretory responses in normal rat distal colonic crypts, there is no convincing evidence that Cl⁻ secretion contributes to the pathogenesis of diarrhoea in these two colitides. In active ulcerative colitis, the inflamed colonic mucosa has the features of a “sick” epithelium, with an increased electrical conductance and an enhanced permeability to monovalent ions. In the descending/sigmoid colon and rectum, inflammation results in a notable decrease or loss of the luminal negative transmucosal potential difference, a consequence of both the increase in epithelial permeability and the virtual absence of electrogenic Na⁺ transport. This reflects a notable (>70%) decrease in basolateral Na⁺, K⁺-ATPase activity, and possibly also a defect in amiloride sensitive apical Na⁺ channels. Loss of the luminal negative potential difference from the inflamed colon results in a decrease in passive Cl⁻ absorption. It can therefore be seen that impaired water absorption secondary to impaired Na⁺ and Cl⁻ absorption (rather than Cl⁻ secretion) is a major pathogenic factor in the diarrhoea of acute colitis. It is presently unclear whether inflammation impairs electroneutral NaCl absorption in the human colon, but this seems highly likely as basolateral Na⁺, K⁺-ATPase is also an essential component of this Na⁺ transport process.

Abnormalities in colonic salt and water transport have also been described in microscopic colitis and collagenous colitis. Whether these two conditions should be regarded as related or distinct entities, and whether they occupy a spectrum of colonic inflammatory disorders that includes ulcerative colitis and Crohn’s colitis, remains controversial. The few studies that have been reported in these relatively uncommon diarrhoeal diseases have been done in vivo. In microscopic colitis, mucosal inflammation is diffuse and variable, and associated with decreases in net water, Na⁺ and Cl⁻ absorption, and Cl⁻/HCO₃⁻ exchange. In contrast to ulcerative colitis, where mucosal damage is usually more severe, the mucosa in microscopic colitis has a normal potential difference and the epithelial permeability to Na⁺ and Cl⁻ seems to be decreased. Thus, impaired colonic water absorption in microscopic colitis may be secondary to a reduction in electroneutral NaCl absorption rather than electronegative Na⁺ absorption. In a single patient with collagenous colitis, saline perfusion of the colon revealed net secretion of Na⁺, Cl⁻ and water, a rise in transmucosal potential difference, and increased intraluminal levels of prostaglandin E₂, which suggests that prostaglandin E₂ stimulated electrogenic Cl⁻ secretion may contribute to the watery diarrhoea which is typical of this disease. In the light of these rather limited studies, it is tempting to speculate that the range of electrolyte transport defects seen in microscopic, collagenous, and ulcerative/Crohn’s colitis reflects different stages of an evolving pattern of epithelial transport dysfunction which is manifested maximally in acute ulcerative colitis. Although approaches to the study of human colonic fluid and electrolyte transport are generally unfashionable, new and more detailed studies of this type are required in patients with microscopic and collagenous colitis if we are to unravel the pathogenesis of diarrhoea and develop more effective therapeutic strategies for these diseases.

The ability of glucocorticoid hormones to decrease diarrhoea in patients with ulcerative colitis and Crohn’s colitis is well known, and generally regarded as part of the general improvement in mucosal function that occurs during suppression of the underlying inflammatory process. However, despite the notable decreases in distal colonic and rectal Na⁺, Cl⁻ and water absorption present in patients with acute ulcerative colitis, single doses of hydrocortisone (100 mg) and methylprednisolone (40 mg) administered parenterally increase net salt and water absorption and stimulate transmucosal potential difference after five hours to the same extent as in normal subjects. It would therefore appear that the high doses of glucocorticoids used in the treatment of ulcerative colitis decrease diarrhoea by exerting a direct stimulatory effect on electronegative Na⁺ absorption (and hence Cl⁻ and water absorption), in addition to their more general anti-inflammatory action. The “mineralocorticoid-like” effects of high dose hydrocortisone and methylprednisolone reflect considerable crossover binding to mineralocorticoid receptors, as well as the activation of glucocorticoid receptors. Glucocorticoid receptor activation results in the stimulation of electroneutral NaCl absorption, and the glucocorticoids used to treat inflammatory bowel disease probably stimulate both electronegative Na⁺ absorption and electroneutral NaCl absorption in the distal colon and rectum. In colitic patients with strictly distal disease, it is likely that the reduction in stool frequency and volume also reflects stimulation of predominantly electroneutral NaCl absorption (and consequently, water absorption) in the non-inflamed proximal and transverse colonic segments.

COLONIC RESECTION

There is surprisingly little information available about the effects of segmental resection of the human colon on the ability of the remaining colon to absorb salt and water.
Human proximal colon is the site of maximal intraluminal concentrations of SCFAs, as well as having the greatest capacity for Na\(^+\), Cl\(^-\), and water absorption per unit area (a considerable portion of which is likely to be SCFA dependent), compared with other colonic segments. However, despite these inherent characteristics of the proximal colon, significant diarrhoea is uncommon in patients after right hemicolecotomy if the remainder of the colon is healthy. This raises the possibility that the processes mediating Na\(^+\), Cl\(^-\), and water absorption in the transverse and distal colon and rectum undergo adaptation, as shown in rat distal colon following resection of the proximal segment. As undigested complex carbohydrates and proteins continue to enter the transverse colon after right hemicolecotomy, it is also possible that this segment functions as a neo-proximal colon, generating greater than normal intraluminal SCFA concentration, thereby enhancing salt and water absorption throughout the remaining colonic epithelium. Patients undergoing left hemicolecotomy are even less likely to develop diarrhoea, given that the descending colon and sigmoid colon normally make a considerable portion of which is likely to be SCFA and undigested complex carbohydrates, thereby enhancing salt and water absorption via high enteroendocytic fistula.

Future research
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