Colonic Fluid and Electrolyte Transport 2022

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The colon is the final segment of the vertebrate digestive system, where fluid and electrolyte transport can be modulated to maintain intestinal and whole-body homeostasis. The expression and activity of many ion transporters in the colon are controlled by a complex and delicate homeostatic ion balance, such that hormones (i.e., aldosterone and angiotensin), pathophysiological inhibitors (i.e., Cholera and STa toxins) and diseases (i.e., metastatic changes) prompt surprisingly distinct responses between the proximal and distal colon segments. Modern and classical experimental methods, such as real-time reverse transcription polymerase chain reaction (RT-PCR), Ussing chambers, genome-wide analysis, next-generation epigenetic sequencing analysis, immunocytochemistry, patch clamping, and siRNA, have allowed the localization, characterization, and measurement of ion transporters. The differential expression and regulation of transporters, described using the following methods, is what gives the proximal and distal colon, apical and basolateral epithelial membranes, and crypts and surface cells unique homeostatic functions and responses to various drugs, hormones, and immune factors.

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1. Electrophysiology

Ion transporters on the basolateral and apical membranes of colonic epithelial cells work in parallel to allow transepithelial ion absorption and secretion while maintaining a homeostatic intracellular ionic milieu. Historically, the Ussing chamber, which uses short-circuit current to determine net transepithelial ion movement properties, was the first means of measuring ionic flux. In an Ussing chamber, both sides of the epithelium are typically flanked by identical electrolyte solutions to eliminate possible paracellular ion movement down osmotic or electrostatic gradients. Since the 1950s, Ussing chambers and derivatives of the technology have been used to observe homeostatic, disease state, and drug- and hormone-induced transepithelial ion movement properties of the colon and countless other mammalian tissues [1]. Clarke offers a comprehensive overview of the Ussing chamber methodology and its applications [1].

2. Isotopic Flux

In addition to short-circuit currents, the radioactive labeling of electrolyte solutions emerged as another method to measure transepithelial ion movement in Ussing chambers. Radioactive flux studies begin with an electrolyte solution isolated from its isotopic solution by an epithelium; the nature of the epithelium's ionic transport capabilities

is determined by changes in isotope concentrations in the chambers over time, which is indicative of an ionic flux [1][2].

3. Intracellular Ion Activity and Transporter Measurements

Measuring an ion current through a specific transporter or channel is also possible using the patch clamp and vibrating probe methods [3]. With patch clamping, epithelial cells of interest are isolated and a pipette is then attached to the cell, allowing for “encapsulation” and a detailed evaluation of a single ion channel. The changes in current that are detected via the pipette and patch clamp amplifier are recorded, and the investigator can then set an intermembrane potential and measure current changes that are occurring due to the activation or inactivation of the channel of interest in the native state, and also while adding inhibitors or activators of the channel. Patch clamping, along with the vibrating probe that measures local current densities on a membrane, is membrane-specific and has allowed a mechanistic understanding and dissection of transepithelial ion transport through the analysis of apical and basolateral membrane transporters and channels in isolation [4].

4. Isolated Perfused Crypts

Various fluorescent and radioactive screening methods that allow live monitoring of the intracellular ion concentrations, pH levels, and membrane potentials of cells in isolated colonic crypts have been developed. These methods have been essential in developing a comprehensive understanding of the many secretory and resorptive mechanisms of colonocytes when exposed to a wide variety of experimental extracellular milieu (exposure to cyclic nucleotides, antibiotics, or divalent cation nanoparticles, to name a few). Furthermore, research on isolated colonic crypts using perfusion through concentric pipettes has revealed much about transepithelial electrophysiology since the method’s development by Dr. Maurice Burg [5] and revision by Dr. Rainer Greger and his colleagues [6], as well as by Dr. John P. Geibel and his colleagues [7].

5. The Molecular Characterization

Immunocytochemistry serves as a visual assay for determining on which membrane a particular ion channel protein is expressed, while patch clamping measures conductance across one membrane to characterize and measure the activity of a channel of interest on either the apical or basolateral membrane [4][8]. Both methods have been useful in determining the mechanism of transepithelial electrolyte movement, as these processes involve the membrane-specific recruitment or localization of different ion channels. Apical and basolateral recruitment and localization are not, however, fixed; RT-PCR measurements demonstrate that ion transporter expression levels, localization, and recruitment are readily modified to account for short-term changes in water or electrolyte intake and other luminal conditions that can adversely or positively affect the homeostasis of fluids and salts [9].

Because different gene expression profiles of ion channels and regulators exist across the colonic longitudinal axis, everything from subtle segmental differences in aldosterone sensitivity to differences in the direction of net ion

movement (as is the case for K^+) have been observed between the proximal and distal colons [10][11][12]. Prolactin, for instance, exhibits opposite effects on ion secretion between the proximal and distal colon [13]. Chromatin immunoprecipitation next-generation sequencing (ChIP-Seq) revealed that of 9866 actively expressed genes in the rat colon, 540 are differentially expressed between distal and proximal segments [14]. This would explain the observed site-specific colon pathology (particularly tumorigenesis), differential effects of drugs and hormones, and even different homeostatic functions of the various segments in electrolyte movement [10][14].

There are also significant differences in the gene expression profiles between crypt and surface cells. The ouabain-sensitive H,KATPase, for instance, is localized in crypt cells, while the ouabain-insensitive isoform is primarily expressed in surface cells [15]. The catechol-O-methyltransferase (COMT) enzyme is another enzyme that is particularly concentrated in the apex of crypts [16]. Perhaps the most significant difference between the two relates to the roles they play in facilitating epithelial turnover during extrusion. The base of a crypt exhibits high proliferation and few signs of differentiation; however, proliferation decreases while expression of differentiation markers increases when moving towards the surface [4]. There continues to be some confusion in the literature on the distinction between crypt and surface cells regarding their respective roles in secretion and absorption [4][17][18]. Several patch clamp experiments have already demonstrated that neither secretion nor absorption are exclusive to the crypts or surface cells [4]. It is the dismantling of this misconception that has allowed models that can explain the large volumes of fluid secretion possible in the colon, particularly under disease states such as secretory diarrhea, with the apical surface area of the epithelium contributing to the loss of electrolytes and the associated fluid movement from cells to the lumen of the colon [4].

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