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Intestinal Secretion and Barrier Function; Implication with Muscarinic Cholinoceptor

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Abstract: Two most important physiological functions of intestinal epithelial cells (IECs) are intestinal secretion and barrier function in order to protect the host from invasive microorganisms. Acetylcholine (ACh) is regarded as a central molecule for the regulation of these gut functions. Although, ACh is considered as a classical neurotransmitter, numerous studies report the synthesis and release of ACh from non-neuronal epithelial cells and are believed to regulate gut functions via cholinergic activation. Recently, it is established that IECs express M1 and M3 muscarinic acetylcholine receptors (mAChRs). Although, the role of M3 mAChR-mediated intestinal secretion in Ussing Chamber has been highly established, little is known about M1 mAChR-mediated intestinal secretion and barrier function. Here, we review the current knowledge about the functions of M1 and M3 mAChRs and their downstream signaling in the regulation of intestinal secretion and barrier function. We also discuss the role of mAChRs in IECs under inflammatory conditions.

Keywords: Intestinal Secretion, Barrier Function, mAChR, Inflammation

1. Introduction

The intestinal epithelium represents an interface between the body and external environment. It subserves a physiological imperative to permit the uptake of fluids and electrolytes in addition to various nutrients while restricting the uptake of undesirable substances into the body, such as toxins, as well as limiting the incursion of microorganisms. As such, both the transport and barrier properties of the epithelium are closely regulated [1]. The physiology of these basic ion transport mechanism is crucial for the proper balance and homeostasis of gastrointestinal tract. Intestinal epithelial cells (IECs) are the first host cells to be infected with invasive bacteria, which enter and pass through these cells to initiate mucosal and ultimately systemic infection. Increased intestinal fluid secretion washes away the invasive bacteria and thus acts as a protective host response after enteric infection with invasive bacteria [2]. Excessive intestinal secretion is clinically concerned with many immuno and inflammatory disorders such as inflammatory bowel diseases (IBD), celiac disease etc. [3, 4].

The intestinal barrier is composed of a single layer of columnar epithelium and interepithelial tight junctions, which reside at the apical-most region of the paracellular space. Tight junctions polarize the cell into apical and basolateral regions (fence function) and regulate passive diffusion of solutes and macromolecules [5]. Thus the intestinal barrier serves as the primary defense against a hostile environment within the intestinal lumen [6]. Acetylcholine (ACh) is regarded as a central molecule for the regulation of gut functions. Although ACh is considered as a classical neurotransmitter, numerous studies report the synthesis and release of ACh from non-neuronal epithelial cells [7], where it exerts its auto/paracrine effects via the stimulation of nicotinic or muscarinic acetylcholine receptors (mAChRs). In a previous study, we reported the expression of mAChRs in intestinal epithelial cells, which are involved in the regulation of various downstream signaling [8, 9]. Five subtypes of mAChRs (M1-M5) with difference in tissue distribution and signal transduction have been cloned [10, 11].

mAChRs are metabotropic G-protein coupled receptors that transduce signals by binding with the specific subtype of G-protein [12, 13]. Briefly, odd numbered mAChRs couple to Gq/11 α subunits to activate phospholipase C (PLC), whereas even numbered mAChRs couple to the Gi α subunit to inhibit adenylate cyclase. Activation of the PLC pathway leads to degradation of membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP2) into diacyl glycerol (DAG) and inositol-1, 4, 5 triphosphate (IP3), which, together, lead to activation of protein kinase C (PKC) and increased levels of intracellular Ca^{2+} as the main outcome [7].

In this review we will discuss the intracellular mechanism of the role of mAChRs in the regulation of intestinal secretion and barrier function under normal physiological and pathological condition.

2. Implication of mAChRs for the Regulation of Intestinal Secretion

The ability to secrete and absorb electrolytes and fluids is critical to maintain proper hydration of the intestinal epithelium. Proper hydration provides the aqueous environment necessary for the processes of contact digestion and nutrient absorption and provides surface lubrication to propel intestinal contents aborally. A properly hydrated gut provides the medium for activity of antimicrobial peptides and movement of water into the lumen contributes to host defense through its ability to flush noxious substances and harmful organisms, thus minimizing their contact with the gut wall. Therefore, regulation of water movement is critical from both physiological (i.e. nutrition) and immunological (i.e. host defense) perspectives. If the process of intestinal hydration becomes dysregulated, as during acute intestinal infections or in the context of intestinal inflammation, diarrhea or constipation can result [7, 14]. In order to maintain the homeostatic fluid balance, controlled-regulation of intestinal absorption and secretion is prerequisite. Intestinal absorption is driven by cations mainly by Na^+ and secretion by anions predominantly by Cl^- ions [15]. Here, we review the control of intestinal Cl^- secretion by ACh, drawing attention to the role of mAChR.

ACh is one of the most important biological regulators of intestinal ion transport, which is synthesized by the catalytic conversion of acetyl-CoA and choline to CoA and ACh by choline acetyltransferase (ChAT) [16, 17]. ACh was described as a classical neurotransmitter in the early 1920s [18]. More recently, a distinction has been made between ACh as a neurotransmitter and ACh as a signalling molecule in non-neuronal tissue [19, 20].

Active ion transport is accomplished through the asymmetrical distribution of ion channels, co-transporters and energy-dependent pumps in the plasma membrane of the polarized IECs. The activity of these ion transport molecules can be up- or down-regulated by increasing or decreasing intracellular messengers, typically Ca^{2+} and the cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP).

The molecular pathway of colonic epithelial secretion has been well elucidated [21, 22]. The energy for the process is derived from the activity of a basolateral Na^+/K^+ -ATPase enzyme. The Na^+/K^+ -ATPase pumps Na^+ out of cells, while pumping K^+ into cells. The activity of this pump creates an electrochemical gradient for cation uptake via basolateral $Na^+/ K^+/2Cl^-$ cotransporter (NKCC1), which carry one Na^+, one K^+, and two Cl^- ions into the cell. K^+ is recycled through regulated channels in the basolateral membrane, thereby maintaining the electrical driving force for Cl^- secretion. Thus the concerted activity of these basolateral transport proteins accumulates Cl^- within the cell to create a gradient for its efflux through the apical Cl^- channels.

ACh via G-protein-coupled mAChRs directly regulates epithelial ion transport by stimulating a fast, transient increase in intracellular Ca^{2+} that leads to a transient increase in Cl^- (accompanied by water) secretion across the apical membrane [23].

Study of intestinal secretion has been greatly facilitated by the development of the Ussing chamber [24]. With this technique, passive flow of ions across a tissue or epithelial cell layer is eliminated by balancing electrical, osmotic, hydrostatic and chemical gradients across the preparation, such that only active ion transport as short circuit current (I_{sc}) is measured.

In a previous report, we have shown that IECs express M1 and M3 mAChRs as major subtypes and stimulation of colonic mucosal sheets with ACh resulted in an increase of intestinal secretion, which was canceled under the presence of M3 mAChR antagonist, darifenacin but was rather slightly augmented under the presence of M1 mAChR antagonist, MT-7 [9], suggesting that M3 mAChR robustly participated as a positive regulator of intestinal secretion. Various researchers have demonstrated that ACh and cholinergic agonists stimulate fluid and Cl^- secretion in mammalian gut [23, 25]. However, the involvement of M1 mAChR was ambiguous. Since M1 and M3 mAChRs both share the similar Gq-coupled G-protein for their signal transduction, it is likely to assume that both subtypes could involve in the positive regulation of intestinal secretion. But practically the net effect of M1 was negative rather positive towards the regulation of intestinal secretion. We found that stimulation of intestinal epithelial cells by ACh activates MAP kinase (MAPK) signaling that was canceled nearly completely by the M1 mAChR antagonist MT-7, suggesting that M1 mAChR dominates MAPK signaling [8, 9]. Barrett has proposed that there is a negative signaling pathway in the downstream of mAChR, in which ERK, p38 or JNK [15, 26, 27] is the responsible signaling molecule, uncoupling an agonist-stimulated increase in intracellular Ca^{2+} from the following response of Cl^- secretion. Recently we reported that ACh-induced intestinal secretion was further augmented by the pharmacological inhibition of mAChR-induced JNK phosphorylation in mice [28]. We therefore assumed that mAChRs behave dichotomously in the regulation of intestinal secretion (Fig. 1). The fast transient increase of

mAChRs-induced intracellular Ca^{2+} -dependent intestinal secretion is partially subverted by the activation of MAPK signaling. Although the net effect of mAChR is toward the secretory functions, the partial negative regulation of intestinal secretion by mAChR-dependent MAPK signaling protects gut from the over-secretory diarrhea.

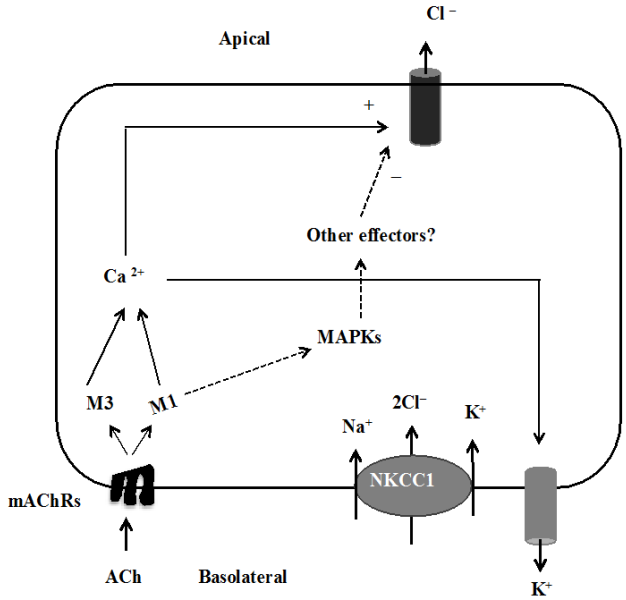

Figure 1. *Schematic diagram for the signaling pathways involved in the positive and negative regulation of Ca^{2+}-dependent Cl^- secretion in IECs. ACh binds to the mAChRs (M1 and M3) on the basolateral membrane of IECs leading to an increase of cytosolic Ca^{2+} that initially stimulates Cl^- secretion via the activation of apical Ca^{2+} -activated Cl^- channels as well as basolateral Ca^{2+} -activated K^+ channels. At the same time stimulation of mAChRs predominantly the M1 subtype activates MAPK signaling that either directly or presumably via some other effectors negatively regulates Ca^{2+}-dependent Cl^- secretion.*

Luminal Intestinal epithelial cells are home for thousands of commensal bacteria as well as are frequently exposed with various pathogenic components that can alter the homeostatic balance of intestinal secretion. Inflammatory bowel diseases (IBDs) including ulcerative colitis (UC) and Chron's disease (CD) are one of the critical inflammatory disorders affecting the gut epithelial cells [29, 30]. The most common symptoms of IBDs are watery diarrhea. In order to study IBDs, various in-vitro animal models have been developed such as trinitrobenzene sulfonic acid (TNBS), dextran sodium sulfate (DSS) or oxazolone-induced-colitis in animal models, which mimics many parameters of IBDs [31, 32].

Recently, we investigated the pathophysiological status of intestinal epithelial cells by adopting TNBS-induced colitis in mouse model [9]. From our observation, we noticed that induction of colitis downregulated mAChRs, in which M1 subtype was highly susceptible to inflammation than M3. We also noticed that there was attenuation of mAChR-mediated MAPKs signaling although the basal phosphorylation of MAPKs was upregulated [9]. These sorts of evidence correlate the uncontrolled regulation of intestinal secretion in IBDs.

3. Implication of mAChRs for the Regulation of Intestinal Barrier Function

It is established that intestinal epithelial cells (IECs) represent an important barrier between lamina propria cells and the potentially harmful luminal contents.

The gastrointestinal tract is the largest of these barriers and is specially adapted to colonization by commensal bacteria that aid in digestion and markedly influence the development and function of the mucosal immune system. However, microbial colonization carries the risk of infection and inflammation if epithelial or immune cell homeostasis is disrupted [33]. The capacity to maintain the segregation between host and microorganism is important for the coexistence of commensal microbial communities and mucosal immune cells. The intestinal epithelium accomplishes this by forming a physical and biochemical barrier to commensal and pathogenic microorganisms.

Increased bacterial translocation due to impairment of intestinal barrier function is one of the critical issues for the risk of developing IBDs [34]. Although it remains unclear whether this is a primary contributor to disease or a consequence of mucosal inflammation, re-establishment of intestinal barrier integrity may be a key to attenuate the disease progression.

Tight junctions (TJs) are the key elements for the development of barrier functions by polarizing the cell into apical and basolateral regions [5]. Focal adhesion kinase (FAK) is one of the important upstream signaling molecules for the modulation of intestinal barrier functions via TJs that is colocalized with TJ proteins, occludin and zonula occludens [35, 36].

Mild form of intestinal epithelial injury commonly occurs in many diseases or in contact with various chemicals, which needs to be repaired rapidly to reform the integrity of epithelial layers. Non-cytotoxic concentration of ethanol causes the disruption of intestinal barrier function leading to increased permeability [8, 36]. The key mechanism of ethanol-induced barrier injury is the internalization of TJ proteins together with the dephosphorylation of FAK. However, ethanol-induced barrier injury can be recovered by the recovery of FAK phosphorylation that subsequently assists the redistribution of TJ proteins into their intercellular membrane [8, 36]. G-protein coupled receptor agonist; CCh enhances the phosphorylation of extracellular regulated kinase (ERK) and FAK [37]. Lesko et al., demonstrated the participation of mAChRs in the tightness of epithelial integrity in the proximal colon [38]. Recently, we report that intestinal epithelial cells express mAChRs [9] and stimulation of mAChRs activates ERK-dependent FAK phosphorylation that rapidly repairs the barrier function after ethanol injury [8] presumably via the redistribution of TJ proteins.

As already discussed before, impaired epithelial barrier function is a common feature of IBDs and is caused, at least

in part, by the elevated level of various cytokines [39, 40]. Treatment of T84 human intestinal epithelial cells with interferon-γ (IFN-γ), one of those cytokines, has been shown to compromise their barrier integrity with a decrease in transepithelial electrical resistance (TER) and an increase in epithelial permeability [41, 42]. Recent reports demonstrated that AMP-activated protein kinase and phosphatidylinositol 3'-kinase is moderately involved in IFN-γ-induced epithelial barrier dysfunction [43-45]. We reported that IFN-γ caused the downregulation of mAChRs that results cholinergic hyporesponsiveness to ERK/FAK and subsequently the attenuation of barrier function [8]. We therefore assumes that mAChRs expressed in IECs participates the positive regulation of intestinal barrier functions via the regulation of its downstream signaling molecules, ERK/FAK and intestinal inflammation causes the downregulation of mAChRs expression resulting the cholinergic hyporesponsiveness to the barrier function (Fig. 2).

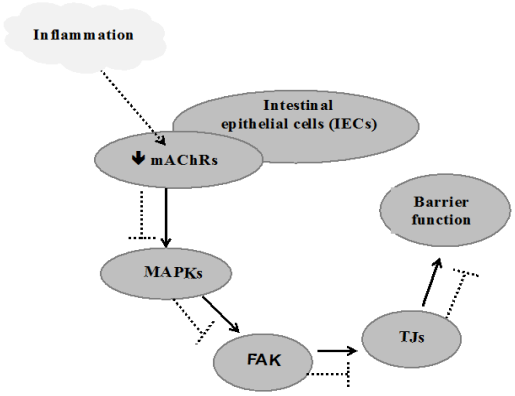

Figure 2. *Schematic diagram for mAChR-mediated signaling pathway involved in the regulation of intestinal barrier function. Stimulation of mAChRs expressed in IECs activates MAPK-dependent FAK phosphorylation that regulates barrier functions via the regulation of tight junctions (TJs) (black arrow). Inflammation causes the downregulation (↓) of mAChRs resulting the impaired regulation of the pathway (dotted line).*

4. Conclusion

Regulation of intestinal secretion and barrier function is crucial for the proper balance and homeostasis of gastrointestinal tract. In this review, we discussed that mAChRs regulates intestinal secretion and barrier function via the regulation of various downstream signaling, which are impaired under inflammatory conditions. Although further studies are necessary to elucidate the complex mechanisms, these results suggest that modulation of mAChRs in IECs could be a good target for the benefits of IBDs.

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