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RGS proteins as targets in the treatment of intestinal inflammation and visceral pain - new insights and future perspectives

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Abstract

Regulators of G protein signalling (RGS) proteins provide timely termination of G protein-coupled receptor (GPCR) responses. Serving as a central control point in GPCR signalling cascades, RGS proteins are promising targets for drug development.

In this review we discuss the involvement of RGS proteins in the pathophysiology of the gastrointestinal inflammation and their potential to become a target for anti-inflammatory drugs. Specifically, we evaluate the emerging evidence for modulation of selected receptor families: opioid, cannabinoid and serotonin by RGS proteins. We discuss how the regulation of RGS protein level and activity may modulate immunological pathways involved in the development of intestinal inflammation. Finally, we propose that RGS proteins may serve as a prognostic factor for survival rate in colorectal cancer. The ideas introduced in this review set a novel conceptual framework for the utilization of RGS proteins in the treatment of gastrointestinal inflammation, a growing major concern worldwide.

Introduction

Communication of complex organisms with the surrounding environment is one of the most significant evolutionary adaptations. It allows these organisms to detect external stimuli and respond to them, which eventually allows them to adapt to changes in the environment. Proper recognition and interpretation of external signals are also crucial for maintenance of organisms' homeostasis. The majority of signals are received by receptors that are first-line components of signaling cascades, initiating transduction of extracellular signals onto

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intracellular effectors, such as enzymes, ion channels, and kinases. One of the most important and ubiquitous groups of receptors with key roles in mammalian physiology are Gprotein-coupled receptors (GPCRs). Over the last few decades GPCRs have become the most successful pharmacological targets, with nearly one third of the pharmaceuticals available on the market aimed at one or more of these receptors[1].

The mechanism by which GPCRs transduce signals into the cell is well-studied and depends principally on the activity of heterotrimeric G proteins. The magnitude and duration of cellular responses caused by external stimuli is determined by the time the G proteins remain activated. Additionally, there are also specific proteins that regulate the speed of G protein deactivation thus affecting GPCR-mediated downstream signaling. These proteins, called Regulators of G protein Signaling (RGS proteins) were discovered more than two decades ago[2–4]. To date, more than 30 mammalian RGS homology proteins have been identified[5], of which approximately 20 regulate the activity of G_q and $G_{i/o}$. Biochemically, RGS proteins act as guanosine triphosphatase-accelerating proteins (GAPs) on α subunits of several types of G proteins (G_q, $G_{i/o}$)[6]. RGS proteins are divided into four subfamilies based on the sequence homology and domain organization, namely R4, R7, R12, and RZ (for a detailed description of each group please see Willars et at.[7]and Wilkie et al.[8]). Expression of RGS mRNAs has been reported in various cell types and tissues, such as neuronal[9], cardiac[10], adrenal[11] and gastrointestinal (GI), including smooth muscle cells[12] and immune cells derived from the gut[13]. Although the majority of RGS proteins modulate GPCR-dependent signaling cascades by a virtue of their GAP activity, several non-canonical actions have also been described, such as regulation of $G_{\beta\gamma}$ signaling, regulation of adenylyl cyclase and regulation of phospholipase Cβ[14]. Several strategies for pharmacological modulation of RGS proteins have been proposed, such as modification of the RGS domain to reduce or increase its GAP activity, allosteric modulation of the non-RGS domain to inactivate the whole protein or blockade of domains necessary for targeting RGS proteins to the plasma membrane[1;15]. Moreover, several small molecule inhibitors/activators of RGS proteins have been reported, that may become leads for drug development or be used as pharmacological tools for studying the modulation of RGS activity in various disease models (please see Table 1 for detailed portfolio of the present-day compounds). In the future the most promising compounds may advance to the clinical testing.

RGS proteins have been documented to regulate the magnitude and duration of signals initiated by several types of GPCRs occurring in the GI tract, including opioid[16], cannabinoid[17] and serotonin (5-HT) [18]receptors. These sites, together with their endogenous and exogenous ligands are involved in maintaining homeostasis, but also in the pathomechanisms of inflammatory GI disorders (e.g. inflammatory bowel diseases, IBD) and visceral pain signaling[19–21]. The prevalence of IBD has reached a high level in developed countries, and continues to rise in previously low-incidence developing countries constituting a major concern worldwide[22]. Considering the common problems associated with pharmacotherapy of IBD, such as loss of responsiveness to drugs by some patients and potential adverse effects of immunosuppressive therapy, the debate on novel anti-IBD treatment strategies is timely and involves a wide target audience.

In this review we analyze the potential of RGS proteins for drug discovery in regard to the pathophysiology of IBD and visceral pain. In principal, we will discuss recent evidence demonstrating that RGS proteins (e.g. RGS 2, 4, 6) affect the function of GPCRs located in the GI tract (Table 2). Particular attention will be given to the opioid, cannabinoid and 5-HT receptors, as their ligands were shown to be crucial modulators of IBD severity. Moreover, efforts have been made to target these systems pharmacologically for future anti-IBD therapies. We also highlight recent advances showing how regulation of RGS protein level/activity may affect immunological pathways involved in the development of intestinal inflammation and explore the possibility that RGS proteins may serve as a prognostic factor in the diagnosis of GI disorders, such as colorectal cancer (CRC). Finally, we refer to the recent reports demonstrating the role of RGS proteins in the development of inflammatory states in extra-intestinal systems, such as kidney and blood vessels, and discuss how this can be translated to the GI tract.

RGS proteins affect specific receptor families in the gut

RGS proteins modulate the activity of endogenous opioid system

Opioid receptors modulate GI tract function in health and disease—In the GI tract, opioid receptors, namely μ, δ and κ (MOR, DOR and KOR respectively) are present in smooth muscle cells and at the terminals of sympathetic and sensory peripheral neurons[21]. Specifically, MOR is expressed in the submucosal and myenteric plexuses and the longitudinal muscle of rat, guinea pig, pig and human intestine[21]. Expression of DOR has been detected in murine esophagus, gastric corpus and antrum, as well as in small and large intestine[23]. KOR is highly expressed in myenteric and submucosal neurons, smooth muscles, blood vessels and mucosa[24]. However, it is also found in immune cells, such as lymphocytes and macrophages[21]. Opioid agonists delay gastric emptying, decrease propulsion in the small and large intestine and enhance absorption of fluids[21]. In clinical conditions, drugs targeting the opioid system are thus used to treat functional GI disorders accompanied by diarrhea or constipation and abdominal pain, such as irritable bowel syndrome (IBS). Moreover, peripherally-acting opioid antagonists are used for the treatment of postoperative ileus[21].

Currently, opioid drugs are not in use for the treatment of IBD. However, experimental data suggest that the stimulation of opioid receptors improves colitis in vivo[25–27]. Animal studies showed that MOR and KOR agonists alleviate intestinal inflammation by reducing the release of pro-inflammatory cytokines, such as TNF-α or IL-6 from macrophages[28]. Furthermore, MOR knockout mice were found to be more vulnerable to inflammation than the wild type animals[29].

RGS proteins constitute a major control point in the opioid signaling—The enhancement of opioid signalling in the gut has great potential to become a new strategy to treat colitis and abdominal pain, as long as it would not be associated with side effects typical for opioids, such as development of tolerance and dependence. We believe that this could be addressed by region-specific RGS proteins.

There is a considerable body of evidence implicating several RGS proteins in the control of opioid receptor signalling. In the nervous system, members of the R7 family, most prominently RGS7 and RGS9-2 determine the sensitivity of MOR and DOR signalling. The R7 family members exist as multi-protein complexes with two auxiliary subunits: R7 Binding Protein (R7BP) and type 5 G protein beta subunit (Gβ5) that are conserved among metazoans. At the molecular level, R7 RGS proteins act to inhibit MOR-mediated regulation of cAMP production, extracellular signal regulated kinase (ERK1/2) phosphorylation and receptor endocytosis[30–33].

Two members of the RZ subfamily, RGS19 and RGS20 have also been shown to play a critical role in the control of opioid signaling. They associate with MOR and regulate responses to MOR agonists, as well as MOR desensitization[34;35].In the synaptosomal membranes RZ proteins may be covalently bound to small ubiquitin-like modifier (SUMO) proteins. These sumoylated proteins associate more efficiently with MOR than DOR. Sumoylation of RZ proteins impairs their ability to function as a GAP on activated G_{α} subunits[36;37]. At the molecular level, RGS20 has been shown to deactivate $G_{\alpha z}$ proteins, what significantly impairs morphine-induced analgesia[35;38].

Several recent reports elucidated the mechanism and effects of an interaction between RGS4 protein and opioid receptors. First of all, it has been shown that RGS4 proteins interact directly with MOR and DOR in a dose-dependent manner [39], and the C-termini of MOR and DOR consist of sequences responsible for coupling of RGS4 and G protein subunits [39].Recently, Yoon et al.[40] reported that pharmacological inhibition of spinal RGS4 reduces inflammatory, but not acute pain responses in mice and this effect is also blocked by non-selective opioid antagonist naloxone. Furthermore, it has been shown that interactions between RGS4 and opioid receptors are bidirectional. For instance, activation of MOR and/or DOR upregulates the expression of RGS4 mRNA, which in turn decreases opioid-mediated signalling [7]. This negative feedback may be blocked by either inhibition, or down regulation of RGS4 protein expression in the gut.

Most recently Papakonstantinou et al.[41] have shown that RGS2 and RGS4 proteins associate with KOR constitutively and upon receptor activation. Moreover, transient expression of RGS2 and/or RGS4 attenuates KOR-mediated inhibition of adenylyl cyclase and phosphorylation of ERK1/2. There is a good amount of evidence to indicate that the activation of opioid receptors, particularly KOR may help attenuating colitis in vivo. Both natural and synthetic KOR agonists produce anti-inflammatory and antinociceptive effects in animal models of IBD[25;26]. Curiously, these effects were more pronounced under pathological states compared to physiological conditions[25]. Thus we suggest that the augmentation of KOR-mediated signalling by pharmacological blockade of RGS2 and RGS4 in the gut could reduce the dose of the primary drugs and/or increase their tissue selectivity. Such a strategy may be free of adverse events typical for opioids, since activation of KORs would not activate the reward system.

Agents targeting RGS proteins can augment opioid-based therapy of GI diseases—It is becoming increasingly clear that multiple RGS proteins contribute to the regulation of MOR, DOR and KOR receptor signalling, likely by controlling different

aspects of receptor signaling. As such, RGS proteins may be exploited in the development of strategies for differential modulation of responses to opioid drugs.

Inhibitors of RGS proteins could be utilized to enhance the effectiveness of synthetic endogenous opioid system (EOS)-targeting drugs, for instance loperamide, acetorphan or trimebutine (Fig. 1A). In some conditions, such as opioid-induced constipation or bowel dysfunction and postoperative ileus, peripherally active opioid receptor antagonists (alvimopan, methylnaltrexone) are used to treat patients. The main task of these drugs is to block the signaling mediated by opioid receptors in the GI tract. In the future, the same goal could be achieved perhaps by upregulating RGS protein expression or activity, specifically in the GI tract, what would uncouple opioid receptor signalling at this location. Such a strategy could be useful for altering opioid signaling selectively in the GI tract, which will allow for lowering of the dose of the drug of interest.

RGS proteins modulate the activity of endogenous cannabinoid system

Endogenous cannabinoid system modulate gut physiology—The endogenous cannabinoid system (ECS) in the gut comprises specific cannabinoid receptors (CB1 and CB2), which are located throughout the GI tract, as well as endogenous cannabinoid ligands. CB1 receptors are abundant in the myenteric and submucosal plexuses of the enteric nervous system (ENS) on excitatory motor neurons, interneurons and intrinsic primary afferent neurons[19;41]. It has been also demonstrated that both types of CB receptors are present in the epithelial and lamina propria mononuclear cells of the human colon[42]. Eventually, dense CB2 immunoreactivity was found in the subepithelial macrophages and plasma cells present in the lamina propria of the colon[43].

CB receptors regulate energy balance and food intake, acting both in the brain and the GI tract. It is also well-established that CB agonists reduce smooth muscle contractility through presynaptic CB1 receptors by the blockade of acetylcholine release from presynaptic membranes (for comprehensive review please see: Izzo et al.[19]).

Enhancement of endocannabinoid signaling in the gut can alleviate intestinal inflammation—In several pathological conditions ECS signalling is enhanced, as indicated by an increased expression of CB1 and CB2 receptors and decreased expression of endocannabinoid degrading enzymes. For example, D'Argenio et al.[44] observed that the level of the endogenous cannabinoid, anandamide (AEA) was two times higher in patients with ulcerative colitis (UC) relative to healthy controls. Interestingly, CB1 receptor-deficient mice are less resistant to colonic inflammation than the wild type animals[45]. Furthermore, indirect activation of CB receptors through inhibition of endocannabinoid-degrading enzyme - fatty acid amide hydrolase (FAAH) also protects against inflammatory stimuli in the colon[46]. These data indicate that the stimulation of CB signaling is a potential strategy for the treatment of IBD, which may be achieved by the inhibition of RGS4 protein (Fig. 1B). Studies show that the expression of RGS4 potently increases in colonic tissue after stimulation with IL-1β, which is one of the major mediators of intestinal inflammation[47;48]. Consistently, it has been shown that RGS4 proteins substantially inhibit AEA signaling at CB1 receptors by their GAP activity[17]. Taken together, these data

indicate that targeting RGS4 either by a small molecule inhibitor or an antibody may lead to an increase in endocannabinoid signaling, which could be spatially limited to the colon. Effectiveness of such a therapeutic strategy may be further increased by a combined treatment with a specific RGS4 inhibitor and the FAAH blocker, which increases the level of endogenous CB agonists.

RGS proteins modulate the activity of serotonin system

5-HT modulate GI tract function in health and disease—Serotonin is one of the most important neurotransmitters in the GI tract. The majority of 5-HT in the body is synthesized and stored in the enteric enterochromaffin (EC) cells located in the intestinal mucosa. In the gut, 5-HT is synthesized by tryptophan hydroxylase 1 (TPH1), which is a rate limiting enzyme in this process[20]. In the neurons, an isoform of tryptophan hydroxylase, TPH2 is responsible for synthesis of 5-HT[49]. Moreover, the 5-HT activity in the GI tract is locally regulated by the 5-HT-selective reuptake transporter (SERT). At least 6 subtypes of 5-HT receptors are distributed on enteric neurons, extrinsic nerve fibers, smooth muscle cells, goblet cells and enterocytes[20]. They can exert excitatory and/or inhibitory activities depending on their location. Serotonin secreted by the EC cells mediates various physiological GI functions including peristalsis, electrolyte secretion and absorption, vasodilatation, as well as perception of pain and detection of gut microorganisms (for comprehensive review, see Mawe et al.[20]).

In the recent years, 5-HT_3 receptor antagonists (5-HT_3RAs) were developed as a highly effective treatment for chemotherapy-induced nausea and vomiting. Moreover, their ability to delay colonic transport in healthy subjects has been employed in the treatment of diarrhea. Consequently, several clinical trials have shown that 5-HT_3RAs (e.g. alosetron, ramosetron, and ondansetron) improve symptoms and quality of life of diarrhea-predominant IBS patients[50–53]. To date, many studies demonstrated that 5-HT signaling is involved in the pathophysiology of inflammatory GI tract diseases, such as Crohn's disease (CD), UC and celiac disease (for review, see Mawe et al.[20]). In animal models, the severity of colitis depends on the concentration of 5-HT in the gut; moreover, TPH1 deficient mice develop less severe colitis in TNBS and DSS models than the wild type[52]. Recently, Li et al.[54] suggested that the pro-inflammatory effect of 5-HT is attributed to the activation of 5-HT receptors on dendritic cells located in the lamina propria of the gut.

The crosstalk between 5-HT and cannabinoid signaling in the gut has been documented to influence visceral pain sensation, which suggests its potential involvement in the pathophysiology of functional GI disorders, such as IBS. Feng et al.[55] have shown that chronic, but not acute stimulation of 5-HT_3Rs with luminal 5-HT increases pain responses to colorectal distention in rats and that these changes correlate with gradually decreasing level of endogenous AEA in the colonic samples. Importantly, treatment with exogenous AEA reduced visceral nociception. Thus, the enhancement of cannabinoid signaling in the colon may reverse the pro-nociceptive effect of increased luminal 5-HT. Although 5-HT_3Rs are not coupled with G_i/G_0 proteins, this study shows that interactions between 5-HT and cannabinoid signaling may play an important role in the pathophysiology of visceral nociception in the gut.

Targeting RGS proteins may weaken 5-HT signaling in the gut and alleviate intestinal inflammation—Recently, Stewart et al.[18] have shown that the endogenous RGS6 is involved in the inhibition of 5-HT signaling and RGS6 deletion selectively enhances the actions of 5-HT at postsynaptic 5-HT_{1A}Rs, and results in anxiolytic and antidepressant phenotype consistent with enhanced serotonergic transmission in the central nervous system (CNS). These observations suggest that enhancers of RGS6 may be used to reduce the 5-HT signalling and reduce its negative effect on intestinal inflammation, providing relief in IBD patients (Fig. 1C). If this could be achieved with high spatial selectivity, e.g. only in the intestinal mucosa, but not in the enteric neurons, a particularly strong anti-inflammatory effect is anticipated. Recently, Margolis et al.[49] demonstrated that reduction of mucosal, but not neuronal 5-HT by administration of mucosal-specific TPH inhibitors at high doses (100 and 200 mg/kg) attenuates inflammation in mouse model of colitis. We thus propose a novel therapeutic strategy based on combined treatment with mucosal-specific TPH inhibitor and enhancer of RGS6 itself or its expression (please see Sjogren et al.[5]for a more detailed description of RGS enhancement approaches). Such a combination may cause synergistic beneficial effect in the GI tract, especially since reduction of mucosal 5-HT does not cause adverse events on physiological motility and secretion.

RGS proteins modulate immunological pathways

IBD is associated with abnormal immunological response, which causes inflammation in the intestinal epithelium. GPCR-mediated signalling is crucial for the activation, proliferation and trafficking of immune cells involved in the development of inflammatory response. T cells, B cells, monocytes, mast cells and neutrophils express GPCRs activated by chemokines and small-molecule inflammatory mediators, such as histamine or leukotrienes. Thus, perhaps not surprisingly, RGS proteins have also been implicated in the regulation of the activity of pro-inflammatory mediators that contribute to the maintenance and resolution of the inflammatory response in IBD (for summary please see Fig. 2). For instance, as shown by Moratz et al.[56] RGS1 expression in B cells inactivates platelet activation factor (PAF) receptor-coupled G protein and decreases the magnitude of its signaling. Hu et al.[47] have demonstrated that Ca^{2+}-dependent contraction of the rabbit intestinal smooth muscle mediated by muscarinic M3 receptors coupled to $G_{\alpha q}$ protein is attenuated by RGS4 through mechanism involving IL-1β. Treatment of colonic muscle cells with IL-1β inhibited acetylcholine-stimulated initial contraction through increased expression of RGS4 mRNA. Since nuclear transcriptional factor κB (NF-κB) signaling is the main pathway activated by IL-1β, it has been suggested that RGS4 is a new target gene regulated by IL-1β/NF-κB signaling[47]. Consistent with these ideas, exposure of rabbit colonic muscle cells to IL-1β in vitro caused an increase in RGS4 mRNA expression, which was blocked by transcription inhibitor, actinomycin D[47]. Up-regulation of RGS4 mRNA by IL-1β was also blocked by selective inhibitors of IκBα or NF-κB activation and in cells expressing the phosphorylation-deficient IκBα mutant (S32A/S36A)[47]. Importance of these findings is highlighted by the key role of IL-1β as one of the main pro-inflammatory cytokines in the pathology of IBD. It further suggests that inhibition of RGS4 protein may alleviate, at least partially, the pathogenic effects of IL-1β in the IBD patients.

Infiltration of immune cells into the site of inflammation is induced by specific chemokines and trafficking of the peripheral T cells to the colonic tissue is one of the most important processes involved in the pathogenesis of IBD[57]. Hence chemokines and their receptors may be modulated pharmacologically to alleviate the intestinal inflammation.

RGS proteins were shown to be involved in the regulation of immune cell trafficking in the body. For instance, it has been reported that B cells carrying a $G\alpha^{i2G184S/G184S}$ mutation in $G\alpha_{i2}$ that renders it insensitive to RGS action lost responsiveness to chemokines[58]. Moreover, the RGS-insensitive mice displayed abnormal serum immunoglobulin profiles, aberrant B cells trafficking, and failure of neutrophils to infiltrate the inflamed tissues[59]. It has also been shown that R4 family members, such as RGS13 and 16 regulate human mast cell and T cell migration and activation[60;61]. Recently, Gibbons et al.[13]showed that RGS1, but not RGS2 and RGS10 mRNA expression is substantially higher in T cells derived from human gut versus peripheral blood. Moreover, they have demonstrated that RGS1 mRNA is significantly elevated in T cells ($CD4^+$, $CD8\alpha\beta^+$) obtained from intestinal samples of patients suffering from CD and UC compared with healthy controls. In the in vitro conditions, transient nucleofection of Jurkat cells with human RGS1 transcripts impaired their migration towards a lymphoid chemokine CXCL12[13]. Similarly, primary human peripheral blood T cells engineered to over-express RGS1 did not align with the gradient of CXCL12. Furthermore, animals injected with colonic T cells from RGS1 knockout mice developed less severe colitis than those receiving T cells from WT colitic mice. In conclusion, this work indicates that RGS1 is a dominant regulator of T cells trafficking in the gut and may thus be involved in the pathology of IBD. These observations suggest new avenues for pharmacological correction of conditions associated with excessive immune cell infiltration and/or activation, such as IBD. Blockade of RGS1 in the gut-derived T cells either by genetic or pharmacological means may have a tremendous therapeutic potential. Thus development of small molecule inhibitors for RGS1 is warranted as an approach to weaken the accumulation of immune cells in the gut.

One of the most exciting emerging concepts is the neurological modulation of inflammatory state in the gut. To date, several in vivo studies evidenced the existence of so called “anti-inflammatory cholinergic pathway” by which anti-inflammatory signals are projected from the CNS throughout the vagus nerve to the intestines (for review please see Bonaz et al. [62]). The key mediators in this process act through GPCRs, e.g. muscarinic receptors. Studies in other systems, e.g. heart resulted in identification of RGS6 proteins as critical regulators of parasymphatetic signaling mediated by muscarinic M2 receptor (for review please see Stewart et al.[63]). Mice lacking RGS6 exhibit increased bradycardia and inhibition of sinoatrial action potential firing[64;65]. Interestingly, RGS4 also plays a role in regulation of the sinus rhythm by inhibition of parasympathetic signaling and modulation of acetylcholine-sensitive potassium current[66]. However, it has been reported recently that RGS6, but not RGS4 is the dominant regulator of heart rate and suggested that RGS4 is masking the influence of another yet unknown member of R7 RGS family that affects heart physiology[67]. It has therefore been suggested that the dysregulation of RGS proteins may contribute to the loss of vagal tone, which is observed in heart diseases and that these proteins consist a potential therapeutic target[63].

It may thus be anticipated that the anti-inflammatory cholinergic signaling within the GI tract also undergoes regulation by RGS proteins. However, there are still several unanswered questions in this field, such as: (i) what types of RGS proteins are the most important in regulation of cholinergic transmission from the CNS to ENS; (ii) what the spatial distribution of these RGS proteins is; (iii) whether these proteins may be targeted pharmacologically to improve intestinal inflammation. This conceptual framework opens up a field for future research aiming at discovery of novel molecular targets for anti-IBD drugs.

Besides their therapeutic potential, RGS proteins may be also utilized as a prognostic marker in GI cancer. A study by Miyoshi et al.[68] revealed that RGS16 is a useful marker for prognosis of CRC. Their analysis of RGS16 mRNA expression in 22 human gastrointestinal cell lines and 124 paired cases of CRC and noncancerous regions showed that RGS16 mRNA is expressed in 17 of 22 human GI cancer cell lines. Moreover, they showed that the expression of RGS16 is significantly elevated in CRC tissue than in corresponding normal tissue at both mRNA and protein levels[68]. Patients exhibiting high RGS16 expression showed worse survival rate than those with low RGS16 expression. These data indicate that high RGS16 expression is a prognostic factor in the course of CRC. It may thus be anticipated that surveying the level of RGS16 in CRC patients will be used in the future prognostic tests for prediction of the disease severity.

RGS proteins contribute to inflammation outside the GI tract

Although this review focuses on the role of RGS proteins in the pathophysiology of inflammatory GI tract disorders, we would like to highlight several recent findings in the extra-intestinal systems, such as kidney or blood vessels for their relevance to the GI tract.

Investigation of the anti-inflammatory role of RGS4 protein in the vasospasm-independent reperfusion injury of the kidney showed that angiotensin II, at a sub-vasoconstrictive doses, stimulated vascular smooth muscle cells (VSMC) to secrete the macrophage chemoattractants (RANTES) more efficiently when the expression of RGS4 was abolished[69]. Moreover, the density of macrophages in the blood vessels after injury was higher in VSMC-specific RGS4 knockout than in wild type. These observations indicate that RGS4 proteins inhibit angiotensin II-mediated cytokine signaling and macrophage recruitment during reperfusion injury in the kidney. Post-ischemic reperfusion injury is one of the central mechanisms in the development of intestinal inflammation and recruitment of macrophages into the site of inflammation is a common feature of all types of inflammatory processes, including colitis. Thus, modulation of expression and/or activity of RGS4 may have additional beneficial effects in managing ischemic colitis.

Another study by Cheng et al.[70] provides evidence that RGS5 is involved in the pathogenesis of atherosclerosis. They showed that mice lacking RGS5 and apolipoprotein exhibited elevated levels of pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β and increased nuclear translocation of Nf-κBP65[70]. RGS5 deficiency enhanced the instability of vulnerable plaques by increasing the infiltration of macrophages and the accumulation of lipids in blood vessels. The authors found that NF-κB and ERK1/2 signaling may be responsible for the effects of RGS5 on atherosclerosis. These results indicate that RGS5

plays a role in stabilization of vulnerable plaques in the blood vessels and prevents their infiltration by immune cells. It is possible that this action of RGS5 could be utilized in other systems where chronic inflammation occurs, such as GI tract. Future studies comparing the expression of RGS5 in healthy and IBD patients are needed to elucidate its potential involvement in the development of intestinal inflammation.

Recently, Shankar et al.[71] investigated the role of RGS16 in the modulation of T helper cells in pulmonary inflammation. They showed that RGS16 controls chemokine-induced T cells trafficking in lungs; consequently, RGS16 knockout animals developed more severe granulomatous lung fibrosis than the wild type. Moreover, T helper 2 and 17 cells accumulated more rapidly in RGS16-deficient mice. These data suggest that endogenous RGS16 exhibits an anti-inflammatory-like activity reflected in inhibition of immune cell infiltration into the site of inflammation. This function of RGS16 requires future in-depth studies in other systems, including GI tract. It may be hypothesized that the protein is downregulated in gut-derived T cells of IBD patients, what may be one of the mechanisms that underlie the pathophysiology of intestinal inflammation.

Conclusions

Discovery of RGS proteins contributed to a better understanding of intracellular signaling mechanisms. Currently, RGS proteins are perceived as key modulators of the magnitude and duration of G protein-mediated signals that are triggered by extracellular stimuli. A growing body of evidence implicates RGS proteins in regulation of GI physiology and pathophysiology (Fig. 3). Future studies on RGS proteins are thus likely to set the stage for important therapeutic advances.

In the early 2000s, many pharmaceutical companies have withdrawn from pursuing RGS proteins as drug targets mainly due to the difficulties in targeting transient interactions between RGS proteins and G_{α} subunits, as well as their undiscriminating nature in interactions with substrates. However, in the recent years novel high-throughput screens for small molecules targeting RGS proteins have been developed and to date several inhibitors of RGS proteins have been evaluated in a biochemical setting. The next critical step appears to be to test their activity in cellular and in vivo models. RGS inhibition should permit spatially specific fine-tuning of signals mediated by several important classes of GPCRs in the GI tract, such as opioid, cannabinoid and serotonin. Novel biologically effective modulators of RGS proteins may also contribute to the development of combination therapies that take advantage of both receptor ligands and RGS modulators, aimed at increasing therapeutic efficacy and/or overcoming side-effects by reducing the dose of compounds targeting GPCR. These strategies may also lead to re-evaluation of drugs that were effective but removed from the market on account to their adverse events. Given these considerations, time is ripe to facilitate the efforts developing small molecules targeting RGS proteins or modulating their expression and/or trafficking.

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Abbreviations

5-HT	serotonin
5-HT$_3$RAs	5-HT$_3$ receptor antagonists
AEA	anandamide
CB1/2	cannabinoid receptor type 1/2
CD	Crohns disease
CNS	central nervous system
CRC	colorectal cancer
DOR	δ opioid receptor
EC	enterochromaffin cells
ECS	endogenous cannabinoid system
ENS	enteric nervous system
EOS	endogenous opioid system
ERK1/2	extracellular signal regulated kinase 1/2
FAAH	fatty acid amide hydrolase
Gβ5	type 5 G protein β subunit
GAP	guanosine triphosphatase-accelerating protein
GI	gastrointestinal
GPCR	G protein-coupled receptor
IBD	inflammatory bowel diseases
IBS	irritable bowel syndrome
KOR	κ opioid receptor
MOR	μ opioid receptor
Nf-κB	nuclear transcriptional factor κB
PAF	platelet activation factor
R7BP	R7 binding protein
RGS	regulators of G protein signalling

SUMO	small ubiquitin-like modifier
UC	ulcerative colitis
VMSC	vascular smooth muscle cells

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Information box 1

The role of RGS proteins in regulation of opioid receptors signaling

- Knockdown of RGS7 and RGS9-2 proteins by anti-sense oligonucleotides enhances potency of morphine analgesia and reduces acute opioid tolerance [72–74]. Similarly, knockout mice lacking RGS9-2 exhibit increased potency of morphine-induced analgesia and reward paralleled by more severe withdrawal symptoms [30;31;75].
- Loss of either R7BP or Gβ5 leads to the increase in behavioral responsiveness to MOR and DOR agonists [74;76;77].
- Knockdown of RGS19 increases the analgesic effect of centrally administered MOR and DOR ligands [34]. Reduction of RGS20 expression by anti-sense oligonucleotides increases supraspinal antinociception elicited by MOR agonists, and knockdown of RGS20 facilitates the development of tolerance to both single and continuous delivery of morphine [34].
- Downregulation of RGS4 increases the potency of DOR agonists [78]. The potency of MOR agonists in inhibition of cAMP accumulation is significantly impaired by induction of transient expression or treatment with purified RGS4 [79].
- MOR and KOR agonists induce upregulation of RGS4 mRNA and this effect is blocked by co-treatment with non-selective opioid antagonist, naloxone[80].
- RGS4 knockout mice are resistant to inflammatory pain.

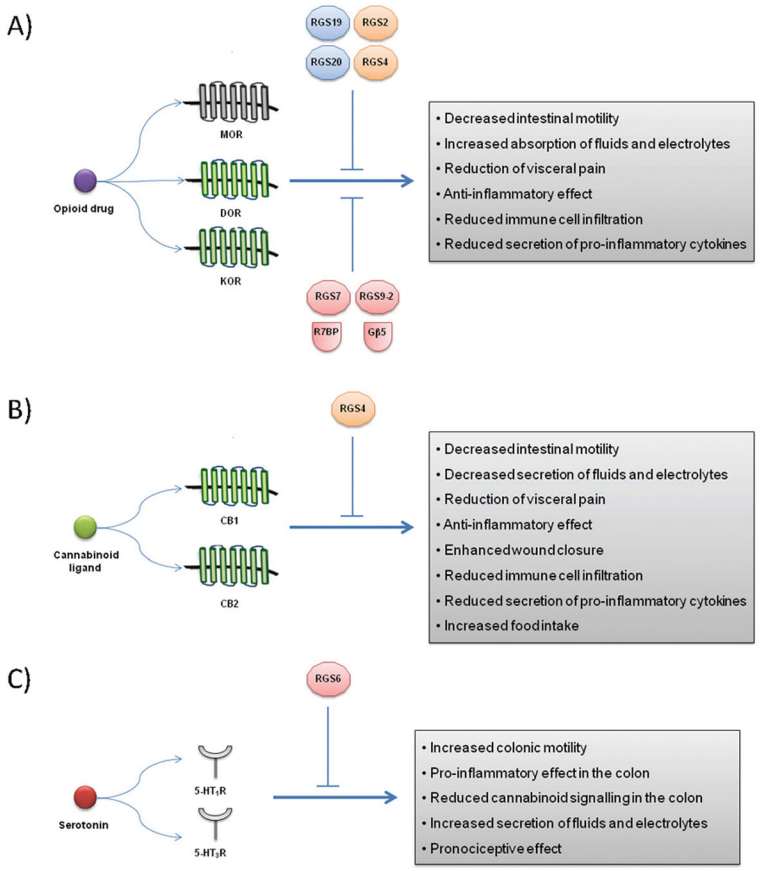

Figure 1.

RGS proteins involved in the modulation of different receptor systems outside the GI tract that may potentially affect the therapeutic effects of opioid, cannabinoid and serotonergic drugs. (A) The influence of RGS proteins on the opioid-induced effects in the GI tract. (B) Possible involvement of RGS4 in the modulation of cannabinoid-mediated beneficial effects in theGI tract. (C) RGS6 as a negative modulator of deleterious effects of serotonin in the GI tract.

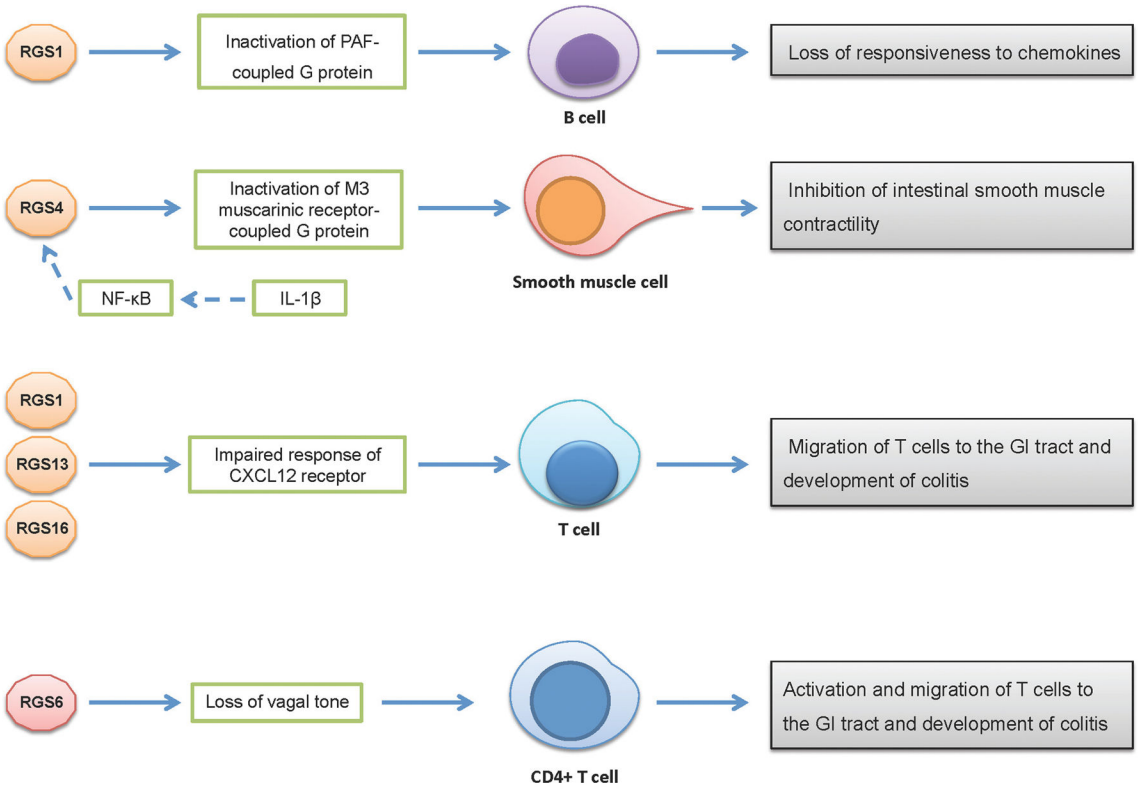

Figure 2.

RGS proteins regulate immune pathways involved in GI pathologies.CXCL12 - chemokine (C-X-C motif) ligand 12; PAF - platelet activation factor.

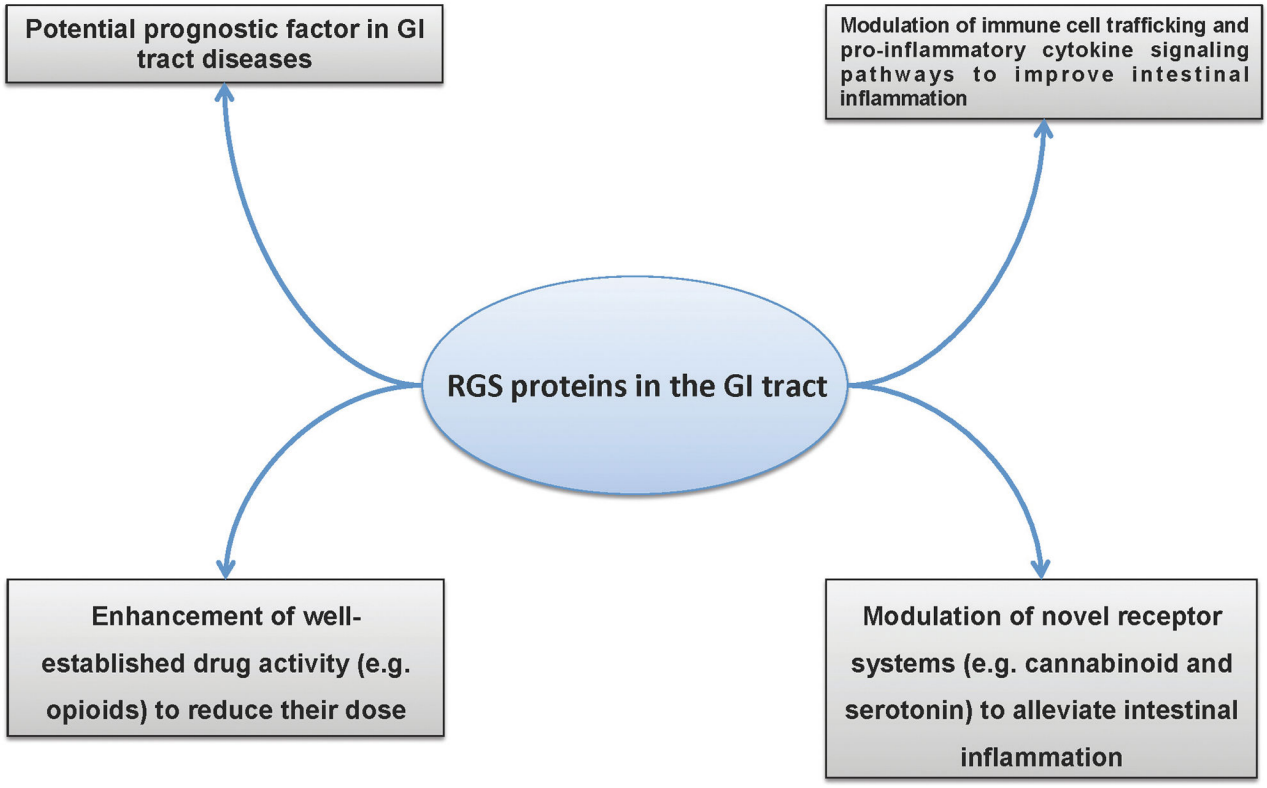

Figure 3.
A scheme illustrating the potential use of RGS proteins as a novel target in the GI tract diseases.

Table 1

Overview on the small molecule inhibitors of RGS proteins.

Name	Chemical name/type		Commentary	Reference
BMS-192364 BMS-195270	1,3-diaryl 1,2,4-(4*H*)- triazol-5-one derivatives	Targeting the G_{α}-RGS interaction to produce an inactive G_{α}-RGS complex.	These compounds cause defective neuromuscular function in the egg-laying process in *C. elegans* and reduction of rat bladder muscle contraction ex vivo.	[81;82]
CCG-63802 CCG-63808	2-hydroxy-9-methyl- 4*H*-pyrido[1,2-α] pyrimidin-4-one derivatives	Targeting allosteric site of RGS4 to weaken its interaction with $G_{\alpha o}$.	These compounds retain activity under reducing conditions and are reversible in the 10-min time range.	[83]
CCG-4986	methyl- *N*-[(4- chlorophenyl)sulfonyl]-4-nitro- benzenesulfinimidoate	Inhibition of RGS4 function through covalent binding to two distinct Cys residues located either near the RGS/G_{α} interaction surface or on the opposite allosteric site.	This compound works in an indiscriminative fashion and does not affect the GPCR signalling in intact RGS4-transfected cells due to its sensitivity to the reducing environment present inside the cells.	[1;84– 86]
CCG-50014	{4-[(4- fluorophenyl)methyl]-2-(4-methylphenyl)- 1,2,4-thiadiazolidine- 3,5-dione}	Covalent binding to the RGS4 allosteric regulatory site.	This compound is active in the nanomolar concentrations and is >20-fold more selective towards RGS4 over other RGS proteins. Moreover, it enhances opioid-mediated analgesic effect in the mouse formalin test.	[87;88]
Compound 11bCompound 13	CCG-50014 derivatives with different side chains attached to N2 and N4	Covalent binding to the RGS4 allosteric regulatory site.	These compounds have improved activity and solubility and display substantial selectivity towards RGS4 over RGS8. Moreover, these compounds lack the off-target calcium mobilization activity observed for CCG-50014.	[89]
Peptide 5nd	Tyr-Trp-c [Cys-Lys- Gly-Leu-Cys]-Lys-NH2, S-S)	Targeting RGS4-$G_{\alpha o}$ interaction. Forming an adduct of one peptide per RGS.	May be selective towards RGS4 and RGS8 over RGS7.	[90;91]

GPCR - G protein-coupled receptor; RGS - regulator of G protein signaling

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Table 2

Involvement of RGS proteins in the pathophysiology of gastrointestinal diseases.

RGS protein	Disease model/experimental setup	Observation/effect	Reference
RGS1	Stimulation of mouse macrophages with LPS	Increased expression of RGS1 mRNA	[92]
RGS1	Intestinal tissue of CD and UC patients	Elevated expression of RGS1 in intestinal T cells	[13]
RGS2	Stimulation of human T cells with IL-2	Decreased RGS2 mRNA expression	[93;94]
RGS4	Stimulation of rabbit intestinal smooth muscle cells with IL- 1β	Increase of RGS4 mRNA expression and attenuation of Ca^{2+}-dependent smooth muscle contraction	[47]
RGS5	RGS5 deficient mice	Increased infiltration of macrophages and expression of pro- inflammatory cytokines in the blood vessels	[70]
RGS16	Human CRC samples	Decreased overall survival in high- expression patients	[68]

CD - Crohn's disease; CRC - colorectal cancer; IL-1β-interleukin 1β; IL-2 - interleukin 2; LPS - lipopolysaccharide; RGS - regulator of G protein signaling; UC - ulcerative colitis.