Annals of Gastroenterology (2014) **27,** 200-206

Main ion channels and receptors associated with visceral hypersensitivity in irritable bowel syndrome

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Abstract

Irritable bowel syndrome (IBS) is a very frequent functional gastrointestinal disorder characterized by recurrent abdominal pain or discomfort and alteration of bowel habits. The IBS physiopathology is extremely complex. Visceral hypersensitivity plays an important role in the pathogenesis of abdominal pain in both *in vitro* and *in vivo* models of this functional disorder. In order to obtain a general view of the participation of the main ion channels and receptors regarding the visceral hypersensitivity in the IBS and to describe their chemical structure, a literature review was carried out. A bibliographical research in the following electronic databases: Pubmed and Virtual Library in Health (BVS) was fulfilled by using the search terms "ion channels" "or" "receptors" "and" "visceral hypersensitivity" "or" "visceral nociception" "and" "irritable bowel syndrome". Original and review articles were considered for data acquisition. The activation of the ATP ion-gated channels, voltage-gated sodium (Na_v) and calcium (Ca_v) channels, as well as the activation of protease-activated receptors (PAR2), transient receptor potential vanilloide-1, serotonin, cannabinoids and cholecystokinin are involved in the genesis of visceral hypersensitivity in IBS. The involvement of ion channels and receptors concerning visceral hypersensitivity is noteworthy in IBS models.

Keywords Visceral hypersensitivity, ion channels, irritable bowel syndrome

Ann Gastroenterol 2014; 27 (3): 200-206

Introduction

Irritable bowel syndrome (IBS) is part of the functional gastrointestinal (GI) disorders (FGID). These are defined as variable combinations of chronic and recurrent digestive symptoms with no related pathologic abnormality and no metabolic or biochemical irregularities. According to the Rome III consensus, IBS is defined by the presence of continuous or recurrent abdominal pain or discomfort relieved by evacuations, associated to alterations of bowel habits [1].

The pathophysiology of the IBS is extremely complex. Nowadays, the proposed mechanisms encompass genetic variables, alterations in bowel motility and visceral sensitivity, psychosocial factors in addition to inflammatory and infectious aspects [2-4].

Regarding visceral nociception, it is observed as hypersensitivity that can be defined as reduced pain threshold and abdominal discomfort, as mentioned by the patients [5]. Though the visceral hypersensitivity physiopathology is not clearly defined, several mechanisms have been proposed, such as those of inflammatory nature, participation of psychosocial factors and alterations of sensory-motor function of the digestive tract, to which a relevant role is attributed regarding the peripheral and central sensitization of the afferent visceral neuron pathways [6].

For better understanding of the increase of visceral sensitivity in the IBS, a broader knowledge of the receptors and ion channels involved in the visceral pain is necessary (Fig. 1). In order to obtain a general view of the participation of the main ion channels and receptors regarding visceral hypersensitivity in IBS and to describe their chemical structure, a literature review was carried out.

The sensory innervation of the digestive tract organs originates from the vagus nerve and primary afferent nerve endings of the spinal cord involving the thoracic-lumbar and lumbar-sacral segments [6,7]. There are three types of afferent fibers: myelinated Aβ fibers that detect innocuous stimuli; myelinated Aδ fibers and non-myelinated C fibers that transmit nociceptive stimuli [6].

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Conflict of Interest: None

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Received 20 November 2013; accepted 8 January 2014

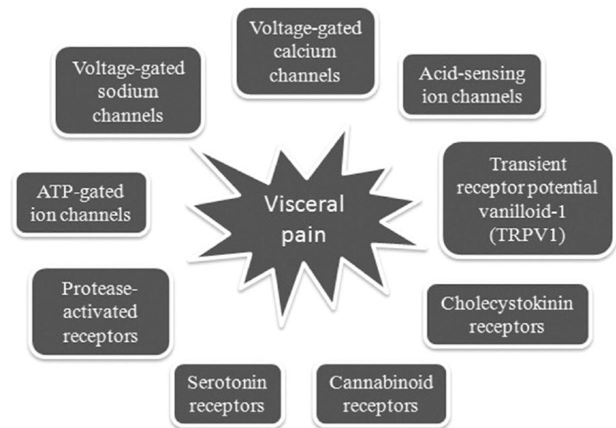

Figure 1 Receptors and channels involved in visceral pain

C fibers can be subdivided into two groups, according to the histological markers. The first group contains P substance and the calcitonin gene-related peptide (CGRP) regulated by the nerve growth factor (NGF) acting on the *tyrosine kinase A receptors* (T*rkA).* In the other group, $P2X_3$ purinergic receptors expressions are regulated by glial cell-derived neurotrophic factor (GDNF). Both groups responded to several types of nociceptive stimuli, including thermal, mechanic and chemical ones, and transient receptor potential vanilloid 1 (TRPV1) receptor was expressed is most of them [6,8].

When the peripheral sensitization occurs, the threshold of nociceptor activation is reduced and its excitability is increased. The change of signaling can be mediated by neuronal ion channels triggered by the neurogenic inflammatory process, involving the release of afferent sensory mediators, nerve lesion, immunologic mechanisms or by recruitment of silent nociceptors activated after cell damage [9,10]. The inflammatory process can sensitize these nociceptors modifying and changing the expression of several ion channels [6].

Adenosine triphosphate (ATP)-gated ion channels

ATP-gated ion channels are multimeric ionotropic receptors and have been identified in afferents nerve endings of the large intestine. These channels are probably responsible for the nociception mediation derived from other pathological conditions of the digestive tract, such as inflammation, infection and cell lesion [6]. There are two types of receptors: the ATP-gated P2X, also known as purinergic; and the G-protein coupled P2Y. Visceral hypersensitivity can be also triggered by the activation of purinergic receptors by means of ATP action released by intestine cells, when colon bloating occurs [11].

A group of researchers carried out a study with induction of chronic visceral hypersensitivity in rats, observing an increase in $P2X_3$ receptor expression, a subgroup of P2X receptors in these animals' colons. It was suggested that the $P2X_3$ receptors are of relevance in the mediation of visceral hypersensitivity, consequently being a potential therapeutic target for IBS treatment [12].

In another study with the use of intracolonic zymosan that causes hypersensitivity in the absence of inflammation in rats, it was verified that the peripheral and central $P2X_3$ receptors are important for determining the increase in colonic sensitivity [11].

Research using $P2X_7$ receptors, subgroup of P2X receptors, located in the macrophages, demonstrated that they were noteworthy mediators of pain and inflammation through the interleukin *(*IL)-1b regulation and release. In a study with animal carriers of post-infectious IBS, it was observed that these receptors play a substantial role in regard to intestinal inflammation, also triggering the development of visceral hypersensitivity [13].

Voltage-gated sodium channels

Voltage-gated sodium channels (Na_v) are members of the ion channels superfamily. They present ten functional molecules located in the central and peripheral nervous system with relatively similar properties [14]. These channels are composed of α subunit in the pore-forming region and, at least, one auxiliary β subunit. The β subunits are multifunctional: they modulate the channel port, regulate their expression level and the way the cell adhesion molecules (CAMs) interact with the extracellular matrix (ECM) and the cytoskeleton. The α subunit family of pore-forming region has nine known types named from $Na_V1.1$ to $Na_V1.9$ [15].

A way of distinguishing the two general classes of Na_v channels is by observing their sensitivity to tetrodotoxin (TTX). Not all the α subunits are sensitive to TTX; therefore the sensitive channels (TTX-S) and the resistant ones (TTX-R) to tetrodoxin can thus be distinguished. The nociceptive neurons express both Na_v TTX-R and TTX-S channels. The Na_v channels TTX-S ($Na_v1.1$, $Na_v1.3$, $Na_v1.6$ and $Na_v1.7$) and TTX-R ($Na_v1.8$ and $Na_v1.9$) are involved in the functioning of nociceptors in normal and pathologic conditions [15-17].

In a study with IBS patients to highlight the role of sodium channels in pain sensation, it was realized that the intra-rectal administration of lidocaine reduced both rectal sensitivity and abdominal pain [18]. Whereas in immune-histochemical studies after biopsies of patients with rectal hypersensitivity, it was noticed that $Na_v1.7$ channel immunoreactive nerve fibers increased meaningfully in this group of patients, when compared to the control group [19].

Voltage-gated calcium channels

The voltage-gated calcium channels (Ca_v) are considered the major supply route of Ca^{2+} from the extracellular environment to the cytosol of electrically excitable cells [20,21]. They involve a large multimeric protein complex consisting of a pore-forming α_1 subunit and other smaller subunits (β, α_2, δ and γ). The α_1 subunit is organized in four repeated domains (I-IV) each containing six transmembrane segments in α-helix (S1 to S6). Each domain has a voltage sensor located in the S4 segment that contains positively charged amino acid residues. Transmembrane-associated loops between segments S5 and S6 contains four amino acid residues Glu-Glu-Glu-Glu, forming the ion selectivity filter in the extracellular part of the pore [22,23].

Ten human genes encode the α_1 subunit of Ca_v which are grouped into three distinct subfamilies: Ca_V1 channels (1.1-1.4) conducting L-type currents; Ca_V2 channels (2.1-2.3), conducting N-type, P/Q- and R-currents; Ca_V3 channels (3.1-3.3) conducting T-type currents [20,22].

The role of T-type calcium channel in visceral pain perception, especially in GI tract diseases, is not yet well established. However, the discovery of modulators of T-type channels demonstrates that there is an important pro-nociceptive function performed by the $Ca_v3.2$ subtype compared to somatic pain [24].

A recent study showed that the genetic or pharmacological blockade of $Ca_v3.2$ prevented the development of colonic hypersensitivity in rats in one IBS type. The authors also observed that the increased current density of the T-type calcium channel in visceral nociceptors coincided with the development of colonic hypersensitivity [25].

Other studies found that large quantities of α_1c subunit of long-term voltage-gated L-type Ca^{2+} channels in the circular smooth muscle colon cells were observed in rat samples with post-infectious IBS [26-28]. In knockout mice lacking the α_1c of L-type Ca^{2+} channels, the amplitude of spontaneous colon contractions is reduced, but not its frequency [29]. These pieces of evidence prove that the calcium L-type channels play a critical role in the amplitude of the intestine contraction.

It appears that α_1c subunit expression can be modulated both positively and negatively, according to the type of pathogenic stimuli and probably also to the type of muscle cells.

Recent studies have shown that trauma such as neonatal maternal separation induce colonic motility dysfunction associated with increased regulation of L-type Ca^{2+} channels in the colon smooth muscle. It has been found that the increased Ca^{2+} influx into smooth muscle cells of the colon of rats subjected to stress is associated with up regulation in the expression of a_1c subunit of L-type Ca^{2+} channels [30].

High potassium (K^+) concentration causes membrane depolarization of smooth muscle cells resulting in the opening of Ca_v. This results in the influx of extracellular Ca^{2+} and an activation of the contraction mechanism. It has been demonstrated that high K^+ concentration leads to smooth muscle contraction, causing induced depolarization by both Ca^{2+} entry and release, through ryanodine and inositol triphosphate (IP_3) receptors [31].

Acid-sensing ion channels (ASICs)

ASICs are encoded by four genes, resulting in ASIC1 to ASIC4 subunits. ASICs are trimeric proteins and can be made up of different combinations of subunits. With the discovery and subsequent cloning of ASICs, they became the main candidates for the sensor of extracellular protons [15]. These channels may play a role in GI nociception and visceral hypersensitivity, although there is no experimental evidence in humans at the current stage.

Animal studies have shown that in ASIC3 knockout mice, there is a reduced visceral mechanosensitivity compared with the control and ASIC1 or ASIC2 knockout [32]. However, in another study with rats, inducing colonic distension with the use of zymogen, it was observed that both TRPV1 and ASIC3 played an important role in the development of visceral hypersensitivity [33].

Protease-activated receptors (PARs)

PARs belong to the seven transmembrane domain of the G protein coupled receptor family that are activated by cleavage of its N-terminal domain by a proteolytic enzyme [34,35]. Four types of PARs are described as selectively cleaved by distinct proteases. PAR1, PAR3 and PAR4 are cleaved by thrombin; PAR4 and PAR2 by trypsine and tryptase and PAR4 is also cleaved by cathepsin G [36].

These PARs are expressed along the entire GI tract in several types of cells, such as enterocytes, mastocytes, smooth muscle cells, endothelial cells and myenteric neurons [37]. The degranulation of mast cells that occurs in inflammation causes the release of serine proteases. The increase in mast cells infiltration has been reported in patients with IBS. The serine proteases act in the PAR1, PAR2, PAR3 and PAR4 [38]receptors.

A study using colon samples from patients with inflammatory bowel disease found that the PAR1 expression was increased when compared to the control group [39]. On the other hand, other research carried out with colon biopsies of IBS patients revealed an increased level of proteolytic activity when compared to the control group. These IBS patients' supernatants sensitized *in vitro* murine sensory neurons. This outcome was prevented by a serine protease inhibitor or by functionally using neurons without PAR2 receptor. Furthermore, the IBS patients' supernatants induced visceral hyperalgesia in rats; the effect was blocked again by serine protease inhibitors [40].

It was demonstrated that PAR2 plays an important role in the interaction among nerves, immunocytes, mast cells and epithelial cells within the intestine wall. The high levels of luminal proteases found in the colonic content of patients with IBS and ulcerative colitis are able to activate PAR2 to promote higher intestinal permeability and sensitivity [35].

Serotonin (5-HT) receptors

5-HT is an important neurotransmitter in the brain-gut interaction, with 80% of the total body 5-HT located in the GI tract [41]. Approximately 95% of the human body's serotonin is produced and stored in enterochromaffin (EC) cells in the intestinal epithelium. However, small amounts of 5-HT are also present in serotonergic neurons of the enteric nervous system where 5-HT takes part in the slow and fast neurotransmission [42-44].

Serotonin, released by EC cells and platelets, is activated via sodium channel coupled $5\text{-}HT_3$ receptor to trigger enteric motor responses [45]. This neurotransmitter is the main mediator involved in the IBS physiopathology. Changes in its metabolism have been proposed as one of the causes of visceral hypersensitivity [46].

After its release, 5-HT stimulates receptor subtypes such as 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_7 that are expressed in the intestine. The activation of presynaptic 5-HT_4 receptors increases the power of the bowel muscle contraction [47]. 5-HT_3 receptor antagonists have offered some help in alleviating pain of IBS symptoms [48]. The 5-HT_4 receptor agonist tegaserod has shown promising results with symptom relief in constipation IBS symptoms [49]. The 5-HT_3 receptor antagonist ondansetron showed improvement in abdominal pain and evacuation in IBS patients when compared with placebo [50]. Some studies with paroxetine, fluoxetine and citalopram which are selective serotonin-reuptake inhibitors (SSRIs) have shown a satisfactory therapeutic effect for the IBS treatment [51-53].

Cannabinoid receptors

The cannabinoid receptors found in mammals are CB1 and CB2, both members of the superfamily of G protein-coupled receptors. CB1 receptors are found primarily in neurons of the brain and GI tract extrinsic and intrinsic nervous system. The intrinsic neurons are located in the submucosal and myenteric plexuses of the enteric nervous system. These plexuses are composed of primary motor neurons, interneurons and afferent neurons [54], having as one of their functions the neurotransmitter release modulation. CB2 receptors have been identified through immunohistochemical studies in most neurons of the ileum enteric nervous system of mice and in peripheral immune cells [55,56].

Anandamide (N-arachidonoylethanolamine or AEA) and 2-Arachidonyl glyceryl (2-AG) are the main endogenous ligands for the cannabinoid receptors. Anandamide and 2-AG are absorbed from extracellular space through the endocannabinoid membrane transporter located in neurons. Within these cells, anandamide undergoes hydrolysis by fatty acid amide hydrolase producing arachidonic acid and ethanolamine. 2-AG, on the other hand, is degraded by the monoacylglycerol lipase enzyme. The anandamide and 2-AG together with their receptors form the "endocannabinoid system" [54].

The CB_1 receptors of the brain and the enteric nervous system, when activated, decrease GI motility by inhibiting of acetylcholine release and inhibiting of cholinergic and non-adrenergic/*non-cholinergic* contraction of circular and longitudinal muscles of the small intestine [55]. In an experimental study, it was observed that the CB_1 receptors were present in the ileum and colon. Their activation by pharmacological agents resulted in inhibition of intestinal smooth muscle cholinergic contraction [57].

Probiotics have demonstrated therapeutic effect in the treatment of IBS. Certain strains of *Lactobacillus acidophilus*, in an experimental study, led to an increase in CB_2 receptor expression in intestinal epithelial cells when compared to non-treated cells or treated with other bacteria [58], contributing to the restoration of visceral sensitivity. In another study, 77 patients with IBS, abdominal pain and distension were randomized to receive *Lactobacillus salivarius*, *Bifidobacterium infantis* or placebo for eight weeks. The group that received *Bifidobacterium* showed improvement in symptoms and normalization of the relationship between IL-10/IL-12 compared to placebo [59].

Recent research investigated the (AAT) n triple repeat polymorphism in the cannabinoid receptor gene (CNR1) in 162 IBS patients and 423 healthy individuals. The aim of the study was to assess whether CNR1 polymorphism could be associated with IBS. The authors observed that polymorphisms in CNR1 receptor are more common in patients with IBS and that the >10/>10 AAT genotype allele is associated with a high symptom rating but not with its frequency [60].

TRPV1

TRPV channels are so named because when activated, they allow the majoritary influx of positive charges into the cell, generating a transient depolarization called "transient receptor potential" which may or not generate an action potential. These channels are described as tetramers (homo- and heterotetramers). Each monomer has six transmembrane domains whose carboxy- and amino-terminals would be located in the cytoplasmic portion and in the pore forming region between the S5 and S6 segments [61].

The TRP channel superfamily is divided into two groups [62,63]. Group 1 is divided into five subfamilies (TRPC, TRPV, TRPM, TRPN and TRPA) and Group 2 has two subfamilies (TRPP and TRPML) [64]. Group 1 carries a strong homologous sequence. The greater region encompasses six transmembrane segments, including the pore [65]. Group 2 varies since its proteins share a difference in the sequence homology of the transmembrane segments and have a long amino acids loop between the first and the second transmembrane domain [64].

The TRPV1 channel is activated by capsaicin and its analogs, lipids and endocannabinoids. Upon activation, a sensation of burning pain is perceived, along with the release of substance P and CGRP, which trigger the neurogenic inflammation process. TRPV1 are described as polymodal sensors responsive to high temperature (>43°C), low pH (pH<5.9) and inflammatory origin of pain [66].

Immunohistochemical studies in rats have observed that nerve fibers of the large intestine that had TRPV1 were present in the mucosa, submucosa, myenteric plexus and circular and longitudinal muscle layers. These fibers are extrinsic primary afferent in the marrow and contain CGRP, substance P, neurokinin A (NKA) and neuronal nitric oxide synthase (nNOS) [67].

Some studies have shown a high number of nerve fibers containing TRPV1 channels in the colon from biopsies of patients with IBS and inflammatory bowel disease. Patients with IBS showed an increased number of TRPV1-positive nerve fibers compared with their healthy controls [68].

In IBS patients' biopsies, TRPV1 is more expressed in the rectosigmoid region compared to healthy subjects. This expression correlates with the gravity of symptoms in patients with post-infectious IBS. These nerve fibers also showed an increased expression of GDNF (glial cell-derived neurotrophic factor), and Trk-A (neurotrophic tyrosine kinase receptors) [69,70].

Cholecystokinin (CCK) receptors

CCK, gastrin and related peptides include a family of peptide hormones and neuropeptides that perform a wide variety of physiological actions in the GI tract, as well as in the central nervous system [71]. Several studies based on functional, pharmacological and molecular approaches have indicated that the effects of these peptides are mediated by two different receptor subtypes identified as CCK1 and CCK2.

CCK1 receptors are mainly located in the GI tract and in some areas of the central nervous system, while CCK2 receptors are widely expressed throughout the GI tract and brain [71,72]. At the intestinal level, CCK1 receptors have been found in both myenteric neurons and longitudinal smooth muscle, partly responsible for the control of motor functions and pain perception [73,74].

CCK is initially characterized as a 33 amino acid peptide sequence, and is present in a variety of biologically active molecular forms derived from a 115 amino acids precursor molecule (prepro-CCK) [75], such as CCK-58, CCK-39, CCK-33, CCK-22, sulfated CCK-8 and CCK-7, unsulfated CCK-8, CCK-5 and CCK-4 [76]. All of them, as well as gastrin, are closely related peptides and share a common amidated C- terminal tetrapeptide sequence, Trp-Met-Asp-Phe-NH2.

Within the CCK/gastrin family of peptides, the characteristic CCK activity depends on the sulfated Tyr residue at the seventh position. If the Tyr residue is not sulfated, or if another amino acid residue is present at this location, the peptide behaves as a gastrin analogue and loses its CCK potency [77]. The CCK1 has a coupled G protein [78] and leads to Gq/11 signaling pathway, enabling the activation of phospholipase C. Besides that, high concentrations of agonists can also lead to Gs pathway activation [79].

Infusing CCK in IBS patients has induced their abdominal pain supporting the theory that CCK has a pro-nociceptive effect [80]. Other authors have shown that the release of CCK may contribute to intestinal motility alterations in patients with IBS [81]. Other studies have shown an increase in plasma CCK levels, as well as increased responsiveness of the colon for this neurotransmitter in patients with IBS [82,83]. A research in women with IBS (constipation-predominant) stage II, using dexloxiglumide 200 mg/day, antagonist of CCK-1, for 12 weeks, showed an improvement in abdominal pain and discomfort compared to placebo [84].

Concluding remarks

This article, based on a literature review, focused on the involvement of ion channels and receptors in the physiopathology of IBS. The involvement of these molecular components in visceral hypersensitivity in experimental models and patients with IBS is remarkable. However, further studies in humans are required to better assess molecular targets in the pathophysiology of this functional GI disorder.

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