ION CHANNELS INVOLVED IN THE INFLAMMATORY PROCESSES OF THE DIGESTIVE SYSTEM

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Abstract. Inflammation is a well regulated process, which has many common but also specific characteristics in each tissue affected. In the gastrointestinal tract (GI), inflammation is related to some detrimental conditions, such as inflammatory bowel diseases, colitis and even carcinogenesis. To prevent inflammation, both the transport and barrier properties of the GI are closely regulated. Ion channels, as principal modulators of the GI barrier function, operate either as primary sensors of external stimuli, or as ion transporters. Therefore, the function of these proteins enables the digestive system to survey its physical and chemical environment in order to circumvent the effects of the inflammatory processes. Therapeutic interventions that modulate the effects on each ion channel address either affected ion transport systems or molecules that modulate the expression and function of these proteins. This review discusses recent findings that identify the role of ion channels in inflammatory processes of GI and brings some consideration about putative therapeutic applications of their ligands.

Keywords: ion channel, inflammation, gastrointestinal tract.

INTRODUCTION

The inflammatory process is essential for homeostasis but also associates with many pathological conditions such as pain, viral infections, drug abuse and cancer.

In the literature of past decades the inflammatory bowel disease (IBD) was designated for a class of symptoms characterised by abdominal pain, increased bowel motility and even bleeding, depending on the severity of inflammation. (1).

The onset of inflammation relates to innate immune response including secretion of inflammatory cytokines, the tumour necrosis factor alpha TNF-α being the most relevant (2).

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The process requires a tight regulation of the inflammatory gene expression, performed by transcriptional factors (e.g. NF-κB family) which are key regulators of the immune system (3). Following activation of NF-κB the expression of pro-inflammatory cytokines (TNF-α and IL-1β, -6, and -12), cyclooxygenase-2 cell-adhesion molecules (VCAM-1 and ICAM-1), will be up-regulated (4). Hence, inflammation is a multistep process, which produces an early innate immune response consisting of the release of immune-regulatory factors. Moreover, this process requires communication of different cells. A cellular process secondary to inflammation is the migration of neutrophils, monocytes and lymphocytes, from the blood to the affected tissue. The migration of inflammatory cells requires a high level of coordination sustained by intercellular communication.

The pathologies of the gastrointestinal tract (GI) associate with inflammatory processes following cellular perturbations such as oxidative stress, gene mutation or epigenetic changes. Moreover, chronic inflammation inducing secretion of pro-inflammatory cytokines is linked to carcinogenic processes in the GI (5). Recent studies describe cellular mechanisms evoked against pathological inflammation in GI tract secondary to food antigens, pollutants and pathogens exposures. Special consideration was accorded to the development of a specific immune reaction such as increase number of T (Treg) cells (6).

In the GI tract inflammation is often related to the damage of the intestinal barrier. The overall body strategy to protect from these perturbations consists of complex cellular mechanisms. One of them is the balance of ion channels and water across the intestinal epithelium. Reports of the last years have indicated gene mutations and/or transcription of ion channels in diseases or following tissue damage, which have been termed as “channelopathies” (7).

The observation that expression and function of channels from GI tract is up- or down-regulated during the inflammatory processes, sustained the idea that these proteins could be putative targets for therapies in gastroenterology.

In this paper, the expression and function of some channels expressed in the digestive system (e.g. K+ channels, Ca2+ channels, Cl- channels) are briefly reviewed with a particular emphasis on their function in the inflammatory process. In order to reveal possible pharmacological opportunities, putative applications of some recently developed channel ligands are also mentioned.

**INFLAMMATORY PROCESSES OF THE DIGESTIVE SYSTEM**

Because the gastrointestinal tract is the first contact place for various pathogens and food antigens, it contains many lymphoid organs participating to the immune reactions. To defend the GI against antigens the immune system triggers a series of reactions such as activation of anti-inflammatory molecules, recruitment of immune cells and secretion of anti-inflammatory cytokines (8).

The transport of nutrients and ions is achieved through the polarised distribution of membrane proteins (e.g. ion channels, ion pumps) in the plasma membrane of the intestinal epithelium (9). Different cellular mechanisms regulate these transport processes, which in turn regulate the movement of water. The impairment of water
transport is characteristic for dreadful conditions such as diarrhoea (water loss) or constipation and intestinal stasis (water retention) which contribute to bacterial overgrowth. Previous studies report a diminished capacity of GI tissues to respond to protective stimuli either in patients with colitis or in animal models (10),(11). The mechanisms of the pro-secretory response to reduce water loss or the pathophysiological significance of this process are not fully elucidated (9).

One of the best known inflammatory conditions is the primary bowel disease characterised by exacerbation of gastrointestinal motility and the increased sensitization of sensory neurons that innervate the GI tract. The latter strongly relates to alterations of ion channel functions. Initially, changes in channel gene or protein expression were connected with functional deregulations. Recent attention has also focused on post-translational modifications such as protein phosphorylation, cysteine thiol modifications or tyrosine nitration as primary means of altering ion channel function. These functional modifications occur in the absence of changes in protein or gene expression. They represent potential mechanisms affected by oxidative/nitrosative stress that alter the gating kinetics of ion channels.

**POTASSIUM CHANNELS**

Potassium channels form a large group of proteins that rules many physiological functions, such as resting membrane potential, action potential, or neurotransmitter release (12). The intestinal secretion is controlled also by processes in which potassium channels play an important role. Intestinal homeostasis is ensured by opening of the apical K⁺ channels which is a prerequisite for colonic K⁺ secretion (13). Similarly, basolateral K⁺ channels generate the driving force for Cl⁻ secretion via apical Cl⁻ channels. This maintains the negative membrane potential, which is dominated by a K⁺ diffusion potential (14).

Up to now, more than 100 potassium channels have been described. Mammalian K⁺ channel subunits have two, four or six transmembrane segments (TMS) corresponding to three major structural classes and a conserved motif called the P domain (pore domain or K⁺ channel signature), as part of the K⁺ conduction pathway. The inflammatory processes of GI tract is controlled by ATP-sensitive potassium channels (KATP), voltage-activated potassium channels, inwardly rectifying potassium channels (Kir), two-pore-domain potassium channels (K2P) and calcium-activated potassium channels (BKCa) (15).

The ATP-sensitive K⁺ channel (K_{ATP}) is a protein composed of an inwardly rectifying, pore-forming subunit (Kir 6.1 and Kir 6.2) and the sulfonylurea receptor (SUR1 and SUR2). These channels regulate cell excitability of the gastrointestinal smooth muscle.

Previous studies showed that K_{ATP} channel activation determine increased hyperpolarization of tissues exposed to inflammation. The analysis of the channel behaviour showed an increased bursting activity specific to K_{ATP} consecutive to inflammation (16). From all subunits, only the pore forming subunit Kir 6.1 showed enhanced mRNA levels, as observed with quantitative PCR. Therefore, it was stated that only transcriptional regulation of the Kir subunit increases bursting duration in inflammation.
Other studies have analyzed the physiological effects of TNF-α on K⁺ channels (17),(18) but reported contradictory results. Hence, TNF-α increased the outward potassium current density (19), whereas suppressed Kir. However, a more recent study clarified these results showing that inflammatory response regulates Kv1.3 and Kir2.1 channels differentially. In fact, inflammation regulates K⁺ channels through both TNF-α-dependent and –independent pathways in a redundant manner (20).

Previous studies show that ulcerative colitis is connected to hypokalaemia, meaning that K⁺ conductance is decreased in these patients. Apparently, in this condition the function of the basolateral Ca²⁺-activated K⁺ channel is impaired (21).

A common gut disorder, irritable bowel syndrome (IBS) characterized by visceral hypersensitivity was connected to alterations of electrophysiological properties of the voltage-gated K⁺ currents. These channels were studied in subpopulations of colonic dorsal root ganglion (DRG) neurons (22).

**CALCIUM CHANNELS**

Ca²⁺ is an intracellular second messenger with versatile functions in many cell types by regulation of multiple cellular processes including cell cycle, apoptosis and proliferation. In resting cells, the cytosolic Ca²⁺ concentration ([Ca²⁺]c) is maintained a low level (50–15 nM). However, this basal concentration is subjected to tremendous changes when cells are challenged to external stimuli such as hormones, growth factors and antigens. Elevation of ([Ca²⁺]c), is consecutive to mobilization of Ca²⁺ from intracellular stores: endoplasmic reticulum (ER) and mitochondria. The intracellular Ca²⁺ concentrations are controlled by the fine-tuned balance between the processes of uptake and extrusion of Ca²⁺ in and out of cells.

A constantly increasing number of Ca²⁺ channels is reported in the literature. These channels include voltage-operated channels (VOCs), receptor-operated channels (ROCs), second messenger-operated channels (SMOCs) and Ca²⁺ release-activated Ca²⁺ (CRAC) channels. Moreover, Ca²⁺ is extruded through molecular transporters, Na⁺–Ca²⁺ exchangers and plasma membrane Ca²⁺-ATPase (23). As Ca²⁺ seems to participate in regulation of every cellular process, many studies advanced the problem of Ca²⁺ channels function in the setting of gastrointestinal inflammation. For instance an isoform of an L-type Ca²⁺ specific to the smooth muscle showed changes in protein as well as of gene expression following inflammation.

Much information comes from studies of colonic inflammation characterised by abnormal motility and consecutively setting of diarrhoea or constipation specific for Crohn's disease. Many cellular factors (e.g. down-regulation of receptors) were shown to be connected with intestinal dysmotility in Crohn's disease (24).

In colonic muscle cells during the inflammatory process Ca²⁺-channel current density is decreased without changes in the expression of L-type Ca²⁺ channel (25). The authors of this study claim that the dysfunction could be improved by inhibitors of NF-κB used for Crohn's disease (25). For instance pyrrolidine dithiocarbamate (PDTC) or sulfasalazine partially but significantly attenuated contractions in rats with induced colonic inflammation. The expression of cytokines such as IL-1β in both rat and mouse colon were significantly changed in the acute phase with inflammation, but no correlations are reported for the chronic stage of the inflammatory process.
The L-type Ca\textsuperscript{2+} channels have a central role in muscle contraction and are required for expression of differentiation genes in smooth muscle (26). Previous studies showed the attenuation of the smooth muscle Ca\textsuperscript{2+} currents as a consequence of dysfunctions in Ca\textsuperscript{2+} channel phosphorylation, whereas decreased protein expression was not altered. These processes were studied in mouse colonic inflammation (27).

New studies show that in colonic inflammation the family members of src tyrosine kinase protein are essential for the activation of the immune system. Previous reports demonstrated that the tyrosine kinase cascade modulates muscle L-type Ca\textsuperscript{2+} channels (28). Particularly c-Src kinase modulates calcium channels from smooth muscle and decreases the motility during colonic inflammation (29). Moreover, changes in gene expression in inflammatory bowel diseases are connected to the altered gating process of the Ca\textsuperscript{2+} channel (27).

The inflammatory bowel disease is mainly characterised by infiltration upon activation of CD4\textsuperscript{+} T lymphocytes into the mucosal layer. The resulted inflammation corresponds to the development of a specific immune-pathology which matches abundant transcripts for IL-2 and IFN-\gamma. In this complex process the initial step appeared to be linked to Ca\textsuperscript{2+} signalling and in consequence to Ca\textsuperscript{2+} channels. Indeed, Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+} (CRAC) channels were related to the T cell activation. In this context, special attention was given to a CRAC inhibitor Synta 66 (GSK1349571A) which down-regulated cytokine genes participating to T cell activation (30).

All overall, the studies discussed above indicate that a better understanding of these processes will pilot valuable insights into the potential therapeutic approaches in inflammatory GI tract conditions.

**TRANSIENT RECEPTOR POTENTIAL CHANNELS**

In the last decades the description of a novel super-family of channels called “transient receptor potential” (TRP) so designated after the role in Drosophila phototransduction, improved the knowledge of the molecular mediators participating to Ca\textsuperscript{2+} entry into cells. A tremendous amount of data burst in the late 10 years and situates TRP channels on the top list of drug targets for various diseases. These proteins were lately reviewed in many circumstances as sensors for taste, temperature, hazardous substances, inflammation and pain.

With the exception of TRPM4 and TRPM5, all TRP channels are permeable to Ca\textsuperscript{2+}. Therefore, TRPs along with other Ca\textsuperscript{2+} channels, mediate changes in intracellular Ca\textsuperscript{2+} concentrations. It is now known that TRPs may be expressed in the endoplasmic reticulum being so an alternative pathway for Ca\textsuperscript{2+} release from organelles (31).

TRP super-family can be divided into seven subfamilies based on amino acid homology (31). The canonical (TRPC) subfamily, the TRPM (melastatin) subfamily, the TRPV (vanilloid) subfamily, the TRPN subfamily (NOMP, No mechanopotential) and the ankyrin family of TRP channels (TRPA).

In the gastrointestinal tract the TRPs have an important role as sensors and transducers of a large variety of chemical stimulus either agreeable such as spices
or unsafe such as and toxins. Besides from this sensory function, TRP channels in the gastrointestinal tract control the membrane potential and excitability of epithelial, muscle, neuron cells or Cajal interstitial cells (ICC). Moreover, they participate to the transport of important ions (e.g. Ca\(^{2+}\) and Mg\(^{2+}\)) and govern motor activity, secretion and are even determinants in the development of GI cancer (32).

TRPV1 channels are sensitized by several spices, noxious heat, and many endogenous stimuli. Moreover, TRPV1 contributes to salt, bitter or metallic taste perception. Furthermore, TRPV1 has a nociceptiv role, relates to pain and hyperalgesia being responsive to many proalgesic factors (33). The most important role of TRPV1 in inflammation is its capacity to integrate thermal and chemical stimuli as a sensor of thermal hyperalgesia that occur during inflammation that leads to the sensation of noxious heat (31).

Co-localized in many afferent neurons of peripheral nerve fibers TRPV1 and TRPA1 may interact in the sensory function (34). Up-regulation of TRPA1 in the intestinal nervous and immune systems is associated with colitis (35). TRPA1 deletion ameliorates pancreatitis and diminishes pain behavior consecutive to pancreatitis (36).

Importantly, antagonist of TRPA1 (e.g. HC-030031) are in the pipeline as therapeutic drugs (37) and combinations of TRPA1 and TRPV1 blockers are considered for superior effectiveness (34).

Another member of the TRP family, TRPV4 was found in colonic nerve fibers from patients with inflammatory bowel disease, being responsible for the mechanosensory responses of colonic serosal and mesenteric afferents (38). TRPV4 agonists and inhibitors (e.g. small molecules RN-1734 and RN-1747) are considered for their therapeutic usefulness and lack of side-effects (39).

Taken together, the experimental findings attribute to TRP channels complex patho-physiological roles in inflammation, pain, and hyperalgesia.

**CHLORIDE CHANNELS**

Along the gastrointestinal tract many chloride channels are expressed participating to the secretion of fluids. A large variety of signaling pathways by which intestinal chloride secretion is modulated have been reported. The regulation of chloride secretion is accomplished by signaling processes, in which cAMP, cGMP, or calcium, are mostly involved.

Epithelial chloride secretion is characterized by a small and transient efflux of Cl\(^{-}\), which is essential for the accurate function of GI tract. Therefore, understanding the key regulators and the mechanisms of this secretion is in fact related to the knowledge of the normal and pathological physiology of the GI tract (40).

Type-2 chloride channels (CIC-2) are up-regulated in the inflammatory bowel syndrome characterized by abdominal pain and constipation. Phosphorylation of CIC-2 channels intensifies the uptake of chloride ions followed by the efflux of sodium ions and water into the intestinal lumen. This leads to increased fluid volume and intestinal secretion.

Chloride secretion is basically related to many other transporters expressed in the intestinal epithelium. For instance, apical calcium-activated chloride channels
are stimulated by the augmentation of intracellular calcium as a result of muscarinic receptors activation. Active uptake of chloride from the bloodstream to the intestinal lumen depends on the basolateral Na-K-2Cl cotransporter which uses energy provided by basolateral Na-K-ATPase. At the end of this process water and sodium follow passively and paracellularly (40).

Cystic fibrosis transient receptor (CFTR) is one of the members of the ABC proteins that functions as a chloride channel (41). Mutations that diminish the CFTR channel activity cause cystic fibrosis (CF), the most common, lethal genetic disease in Caucasians (42). In CF patients, epithelial cells that normally transport Cl⁻ in airways, pancreas, and other tissues become Cl⁻ impermeable, causing defective salt, water, and protein transport. Moreover, it has been reported that increased activity of the CFTR caused by bacterial toxins results in secretory diarrhoea.

Early studies focused on the investigation of the CFTR role in inflammation established that TNF is a mediator of the inflammatory process (43). The authors observed that the CFTR regulator sequence has 89% homology with the known TNF-regulated collagen gene sequence. It was reported that TNF did not affect the CFTR gene transcription rate, but the stability of CFTR mRNA transcripts, resulting in a reduction by 65% mRNA half-life. Other studies have showed that IL-1β up-regulates the steady-state levels of the CFTR mRNA and protein expression (44) via a protein kinase C (PKC) pathway. Apparently, a PKC isoform might be involved in the mechanism of CFTR inhibition.

Some ligands of the chloride channels have been propose in therapies against the irritable bowel syndrome. For instance, lubiprostone is a local activator of ClC-2 channels with helpful but modest effects on IBS symptoms, whereas crofelemer described as a CFTR inhibitor showed more promising results (45).

**CONCLUSIONS**

In conclusion ion channels participate in many disorders of GI function. Together with other disease, GI inflammation is often associated with specific channelopathies. However, all channels reviewed in this paper are distributed throughout the body and have many important functions outside the GI tract. Therefore, translation of these results to the clinical application and especially discovery of new drugs with target limited to channels expressed in the gastrointestinal tract is a subject of intensive research.

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