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TRPV4: New therapeutic target for inflammatory bowel diseases

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-Abstract-

The transient receptor potential vanilloid-4 (TRPV4) belongs to a family of ion channels and can be activated by warm temperature, hypotonicity, cell swelling or lipid mediators of the arachidonic cascade. The metabolites or events responsible for TRPV4 activation are associated with inflammation, arguing in favor of a role for this receptor in inflammatory diseases. The first studies have focused their attention on the role of TRPV4 in neurons and endothelial cells but TRPV4 cellular distribution is widespread, particularly in the gastrointestinal tract. Herein, we review a number of studies demonstrating the expression of TRPV4 in the gut, the regulation of its expression and functions by inflammatory mediators in that organ and the consequences of TRPV4 activation or inhibition in the intestine. We further discuss the relevance of considering this receptor as a potential target for therapeutic development in inflammatory bowel diseases.

-Introduction-

The transient receptor potential vanilloid-4 (TRPV4) is a member of the osmotic avoidance abnormal family member-9 (OSM9)-like transient receptor potential subfamily, in the transient receptor potential (TRP) superfamily of ion channels[1,2]. This protein is a calcium-permeable, non-selective cation channel that is thought to be involved in the regulation of osmotic pressure[2].

Mutations in the gene of TRPV4 are associated with a vast range of disorders that affect in particular the skeletal and the peripheral nervous systems[3,4,5], but none of those mutations has been associated so far with inflammatory diseases. When considering the type of molecules and events that are capable of activating TRPV4, one can hypothesize that this channel, although it might not be involved in the etiology, could participate to maintain a chronic state of inflammation. TRPV4 is endogenously activated by moderate heat, cell swelling, anandamide, and by different metabolites of the arachidonic acid cascade[6]. All those metabolites and events are associated with or present in inflamed tissues, suggesting that TRPV4 could be activated upon inflammation. Indeed, several studies now report that the expression of TRPV4 is increased in inflamed tissues or that activation of TRPV4 causes signs of inflammation. This has been shown in the joints[7,8], in the paw[9], in the pancreas (for inflammatory pain)[10], in adipose inflammation[11], in inflamed bladder[12], and in the gastrointestinal tract[13,14]. The fact that TRPV4 seems to be implicated in inflammatory responses occurring in different tissues and organs, potentially implicates this receptor as a common mediator that could be viewed as a possible molecular target to treat inflammation.

How can a molecule be defined as a potential target for the treatment of inflammation? Several features should be associated with the molecular signature of this target. First, the cellular or sub-cellular expression of the molecule of interest could be changed upon inflammation. Second, the activation of this molecule should cause several signs of inflammation. Third, the functions of the molecule of interest could be regulated by other inflammatory mediators. Finally, the inhibition of this molecule should relieve from inflammation. Here, we will review the facts demonstrating that TRPV4 responds to all those criteria in the intestine, thereby presenting this receptor, as a potential therapeutic target to treat intestinal inflammation and inflammatory bowel disease (IBD).

-TRPV4 expression in the gut-

The first study reporting the expression of TRPV4 in the gut showed that retrogradely labeled neurons from the gut expressed TRPV4 transcript[15]. Later on, Sipe et al. showed that in intestinal neurons, TRPV4, proteinase-activated receptor-2 (PAR2) and calcitonin gene-related peptide (CGRP) were expressed in the same neurons, suggesting that TRPV4 is present on sensory neurons[16]. Such presence and sensory functions in visceral sensory pathways were confirmed for TRPV4 by the study of Brierley et al.[17]. However, all those studies, like earlier studies investigating the function of TRPV4 on somatic pain, were focused only on neurons. Cenac et al. performed TRPV4 immunostaining in whole colonic tissues in mice, and demonstrated like others, that TRPV4 was expressed on neurons, but also that TRPV4 was strongly expressed in intestinal epithelial cells, and in unidentified cells present in the submucosa and in the muscular layer of the intestine[18]. D'Aldebert

et al. observed that in a mouse model of colitis, the expression of TRPV4 was significantly increased in colonic tissues[13]. In the same study, the authors investigated the expression of TRPV4 in human colonic tissues, demonstrating that the receptor was strongly expressed in human intestinal epithelial cells, but also in glial cells. Investigating the expression of TRPV4 in tissues from IBD patients (Crohn's and ulcerative colitis), the authors identified the presence of TRPV4 in infiltrated CD45-positive cells. A more recent study reported that in tissues from Crohn's disease or Ulcerative colitis patients, TRPV4 mRNA expression was up-regulated compared to tissues from non-IBD patients[14]. Therefore, evidences that TRPV4 is present in intestinal tissues, including human tissues have been raised. The receptor is present there in different cell types: intestinal epithelial cells, neurons, glial cells, and infiltrated inflammatory cells. In addition, the expression of TRPV4 is up-regulated in colonic tissues upon inflammation, suggesting that the receptor is activated in this condition. What are the consequences of TRPV4 activation in intestinal tissues? Could it participate to maintain an inflammatory state?

-Consequences of TRPV4 activation in the gut-

From the discovery of TRPV4, most of the studies were focused on pain pathways, demonstrating that TRPV4 activation was inducing somatic pain[19], but also visceral pain, when TRPV4 agonists were introduced into the colon[18,20]. The first study investigating the effects of TRPV4 activation on several parameters of inflammation was performed after injecting different TRPV4 agonists into the mouse paw[9]. There, TRPV4 agonists induced edema and granulocyte infiltration, in addition to allodynia and hyperalgesia. This study demonstrated that the inflammation induced by TRPV4 agonists was due to a neurogenic mechanism,

involving the release of neuropeptides (substance P and CGRP) by sensory neurons. In the joint, the same features of inflammation were induced after intra-articular injection of TRPV4 agonists[7]. In the gut, activation of nociceptors, as well as visceral hyperalgesia and allodynia were observed after intraluminal administration of TRPV4 agonists[18,20]. Tissue damage characteristic of inflammatory insults were also observed after the intracolonic administration of different TRPV4 agonists. Edema, hyperemia, and important mucus secretion were noticed[13]. These signs of colitis developed from 3 to 6 hours after a single administration of TRPV4 agonist into the colonic lumen. Pro-inflammatory chemokines and cytokines were found up-regulated in mouse tissues: Interleukin-6, keratinocyte-derived chemokine (KC), monocyte chemotactic protein-1 (MCP-1) and Rantes were significantly increased compared to their expression in naïve mice. Inflammatory cells were also found infiltrated into tissues, as observed by a significant increase of myeloperoxidase activity after colonic exposure to TRPV4 agonist. Barrier function was also affected by a single administration of TRPV4 agonist into the mouse lumen. Intestinal permeability to a macromolecule (Cr-Ethylenediaminetetraacetic acid: CrEDTA) was significantly increased in those mice. Repeated intracolonic administrations of TRPV4 agonist caused chronic inflammation that last for several days (up to 8-days)[13]. In contrast to what was observed in the paw, the colitis induced by intracolonic administration of TRPV4 agonist was not mediated by a neurogenic mechanism, since all parameters of inflammation in response to TRPV4 agonist were still present in mice depleted from neuropeptides[13]. This observation was also supported by the fact that intestinal epithelial cells in isolation responded to TRPV4

agonists. Exposure of human intestinal epithelial cell lines to TRPV4 agonists conferred to those cells a pro-inflammatory phenotype, where they released different cytokines and chemokines (interleukin-8, interferon gamma-induced protein-10: IP-10, MCP-1 and monokine induced by gamma interferon: MIG)[13].

Clearly, acute activation of TRPV4 in the gut leads to all the features usually observed in inflammation: swelling, redness, pain (see Figure 1). Chronic activation of TRPV4 maintains all the parameters of colitis. This suggests that if TRPV4 is activated in tissues from inflammatory bowel disease patients, it should participate to maintain inflammatory signs. In the gut, TRPV4 may be activated on visceral neurons, but also on other cell types that could confer a pro-inflammatory phenotype, and in particular intestinal epithelial cells.

-Regulation of TRPV4 by inflammatory mediators in intestinal cells-

In order to define whether TRPV4 could constitute a potential therapeutic target to treat gut inflammation, one important point is to understand how inflammatory mediators could regulate the functions of TRPV4. We have mentioned above how the level of expression of TRPV4 was up-regulated in inflamed tissues, and particularly in inflamed colon, both in animal models and in tissues from inflammatory bowel disease patients[13,14]. When more TRPV4 receptor channels are expressed in the inflamed intestine, signaling through TRPV4 may be more potent if endogenous agonists are present within those tissues. Inversely, anti-inflammatory molecules significantly decreased TRPV4 expression. This was demonstrated in a model of croton oil-induced inflammation of the upper intestine. Treatment with the cannabinoid agonist cannabidiol was able to significantly reduce the expression of TRPV4 in the jejunum and the ileum of mice[21]. Interestingly, in that model of

croton oil-induced inflammation, TRPV4 protein expression was not up-regulated, but cannabidiol treatment significantly inhibited mRNA basal expression of TRPV4 [21].

Not only the expression, but also the cellular biological response of TRPV4 seems to be modified by mediators of inflammation in the intestine. Indeed, studies demonstrated that pro-inflammatory mediators such as histamine, serotonin or proteases were able to potentiate the cellular response to TRPV4 activation. In sensory neurons, the two early mediators of inflammation histamine and serotonin modified cellular responses to TRPV4 activation according to two different profiles. Neurons that did not respond to TRPV4 agonists showed a calcium mobilization response after having been exposed to serotonin or histamine: from silent nociceptors, those neurons became responders to TRPV4 activation. Other neurons that did mobilize calcium in response to TRPV4 agonists, showed a potentiated calcium response after having been exposed to serotonin or histamine[20]. Proteases, through the activation of the pro-inflammatory[22,23,24,25,26] and pro-nociceptive[27] receptor PAR2, were also able to potentiate the cellular response of enteric neurons to TRPV4 stimulation[16,18]. In neurons, potentiation of the cellular response to TRPV4 agonists by PAR2 agonists was shown to be dependent on protein Kinase A (PKA), protein Kinase C (PKC) and protein Kinase D (PKD)[28], while PKC was involved in histamine- and serotonin-induced TRPV4 potentiation[20]. This suggests that sites of protein kinase phosphorylation are present on TRPV4 protein. Indeed, Peng et al. identified on TRPV4, a phosphorylation site at a Serine residue, that was involved in the enhancement of

TRPV4 channel function[29]. Poole et al. also identified a key Tyrosine residue (TRPV4-Tyr-110) that was involved in PAR_2/TRPV4 coupling, and that was required for sustained inflammatory signaling[30]. However, phosphorylation does not seem to be the only mechanism by which pro-inflammatory mediators can sensitize TRPV4 responses. In their study, Cenac et al. also demonstrated that a pool of intracellular TRPV4 receptors was sent to the plasma membrane of neurons after exposure to histamine or serotonin. This translocation of the receptor to the plasma membrane was dependent on the activation of mitogen-activated protein kinase kinase (MAPKK) [20] (Figure 2). Finally, a third mechanism seems to be involved in inflammatory mediators-induced TRPV4 potentiation. This mechanism depends on the activation of Phospholipase A_2 (PLA_2)[20,30]. Cenac et al. demonstrated for serotonin and histamine, that in primary afferents, PLA_2 was activated and involved in TRPV4 potentiation[20], while Poole et al. demonstrated that inhibition of PLA_2 and cytochrome P450 epoxygenase attenuated the PAR_2-induced TRPV4-dependent calcium sustained response[30]. This suggests that pro-inflammatory mediators can generate arachidonic acid-derived lipid mediators that can activate TRPV4. Indeed, 5,6 epoxyeicosatrienoic acid, a metabolite of PLA_2 pathway, activates TRPV4[31], and can be released from sensory neurons. Taken together, these results show that pro-inflammatory mediators can enhance TRPV4 signaling through 3 different possible mechanisms that can occur concomitantly (see Figure 2). First, they can induce the phosphorylation of the receptor channel at the membrane surface and make this receptor more sensitive to its agonists. Second, inflammatory mediators can induce the mobilization of intracellular pools of receptors, thereby increasing the expression

of functional receptors at the cell surface. Third, they can induce the release of endogenous lipidic agonists, which then activate the receptor by autocrine or paracrine mechanisms. Therefore, it is a very potent signal enhancement that could occur for TRPV4 upon inflammation. These mechanisms were demonstrated only in neurons so far, because TRPV4 biological functions were initially studied in neurons. The same potent mechanisms of activation and sensitization could occur in other cells of the intestine that express TRPV4, and in particular in intestinal epithelial cells. Indeed, D'Aldebert et al. reported that intestinal epithelial cells monolayers pre-exposed to PAR1 or PAR2 agonists showed a potentiated response to TRPV4 agonists[32]. In the gut, both PAR1 and PAR2 activation caused pro-inflammatory signals[33,34,35,36], and therefore, can be considered as inflammatory mediators. In contrast, another member of this family of receptors: PAR4, which has been shown to exert opposite effects to PAR1 or PAR2 in many cell systems[37], was not able to potentiate TRPV4 signals in intestinal epithelial cells. PAR4 was able to inhibit TRPV4 agonist-induced calcium signal in primary afferents[38]. Other anti-inflammatory molecules, such as hydrogen sulfide (H_2S)[39,40,41], were able to modulate TRPV4 signaling. D'Aldebert et al. reported that the response to TRPV4 agonists of intestinal epithelial cells pre-exposed to NaHS, was significantly inhibited[32]. TRPV4 functions also seemed to be regulated in the upper gastrointestinal tract. A study reports that TRPV4 was functional in esophageal epithelial cells and that TRPV4 response was modulated by acidic pH[42]. The functional significance of such inhibition is not clear yet. Whether TRPV4 inhibition in this cell type would have protective or damaging effects should be determined.

However, the fact that TRPV4 activation in esophageal epithelial cells led to the production of interleukin-8[43] suggests that in the epithelium of the upper gastrointestinal tract as well, TRPV4 exerts pro-inflammatory functions.

Taken together, the data that have been raised on the regulation of TRPV4 expression and functions, point to an enhancement of TRPV4 signals by pro-inflammatory mediators and an inhibition of this signal by anti-inflammatory mediators. This is in agreement with an active role of TRPV4 in inflammatory responses, and a potential pro-inflammatory function of TRPV4.

-Benefits of TRPV4 antagonism-

In order to fully demonstrate the involvement of TRPV4 in inflammatory processes, studies blocking the expression and/or functions of TRPV4 are necessary. One study reports such approach in the gut. The authors used the RN1734 compound, a reported selective TRPV4 antagonist[44]. They induced colitis in mice by the intracolonic administration of trinitrobenzene sulfonic acid, and they observed that mice treated with the TRPV4 agonist were significantly protected from signs of colitis[14]. This study clearly establishes the involvement of TRPV4 activation in this model of IBD, suggesting that TRPV4 could constitute a promising target for the development of anti-inflammatory therapies in IBD. Another study, although not focused on gut functions, reports that TRPV4 antagonism or deletion in mice protected from diet-induced obesity, adipose inflammation and insulin resistance[11].

-Conclusion-

A number of studies point towards a strong role for TRPV4 in intestinal inflammation and potentially in IBD. Indeed, TRPV4 expression and functions are

modified by intestinal inflammation, the activation of TRPV4 provokes pro-inflammatory signals in the gut, and inhibition of TRPV4 activation is protective in models of colitis. All these data support the concept that TRPV4 should be considered as an interesting target for drug development in the field of IBD. However, a number of questions remain. In particular, it is not clear whether endogenous agonists of TRPV4 are released within intestinal tissues in an IBD context, and therefore could activate the receptor in that pathological condition. Which cell type expressing TRPV4 in the gut would be mostly responsible for the pro-inflammatory effects of the receptor activation? Consequently, which TRPV4-expressing cells should be considered as main targets for TRPV4 inhibition? What would be the potential side effects of TRPV4 inhibition in the intestine? TRPV4-deficient mice are viable and although they demonstrated phenotypes for osmolarity regulation[45], they did not demonstrate lethal phenotypes[6]. Answering all those remaining questions is a pre-requisite to the development of TRPV4-targeted therapies in the gut.

Figure legends:

Figure 1: Consequences of TRPV4 activation in the gut.

Figure 2: Mechanisms of inflammatory mediators-induced TRPV4 regulation. TRPV4 expression and/or functions can be exacerbated (+) by pro-inflammatory mediators (Proteases, Protease-Activated Receptor-2: PAR2, Histamine, Serotonin), according to 3 different pathways: Pro-inflammatory mediators induce (1) TRPV4 phosphorylation by protein kinase A, C or D, this phosphorylation provokes a conformational change towards an enhanced response of the receptor; (2) TRPV4 intracellular pools mobilization to the plasma membrane, through a MAPKK-dependent mechanism ; (3) the production of TRPV4 endogenous agonists, through a phospolipase A_2 (PLA_2)-, cytochrome P450 (CytP450)- or epoxygenase (epox)-dependent pathway, these endogenous agonists could activate the receptor in a paracrine or autocrine pathway. TRPV4 expression and/or functions can be inhibited (-) by anti-inflammatory mediators (Protease-Activated Receptor-4: PAR4-activating proteases, cannabinoids, hydrogen sulfide (H_2S)), through a yet unknown mechanism.

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Figure

Figure 1:

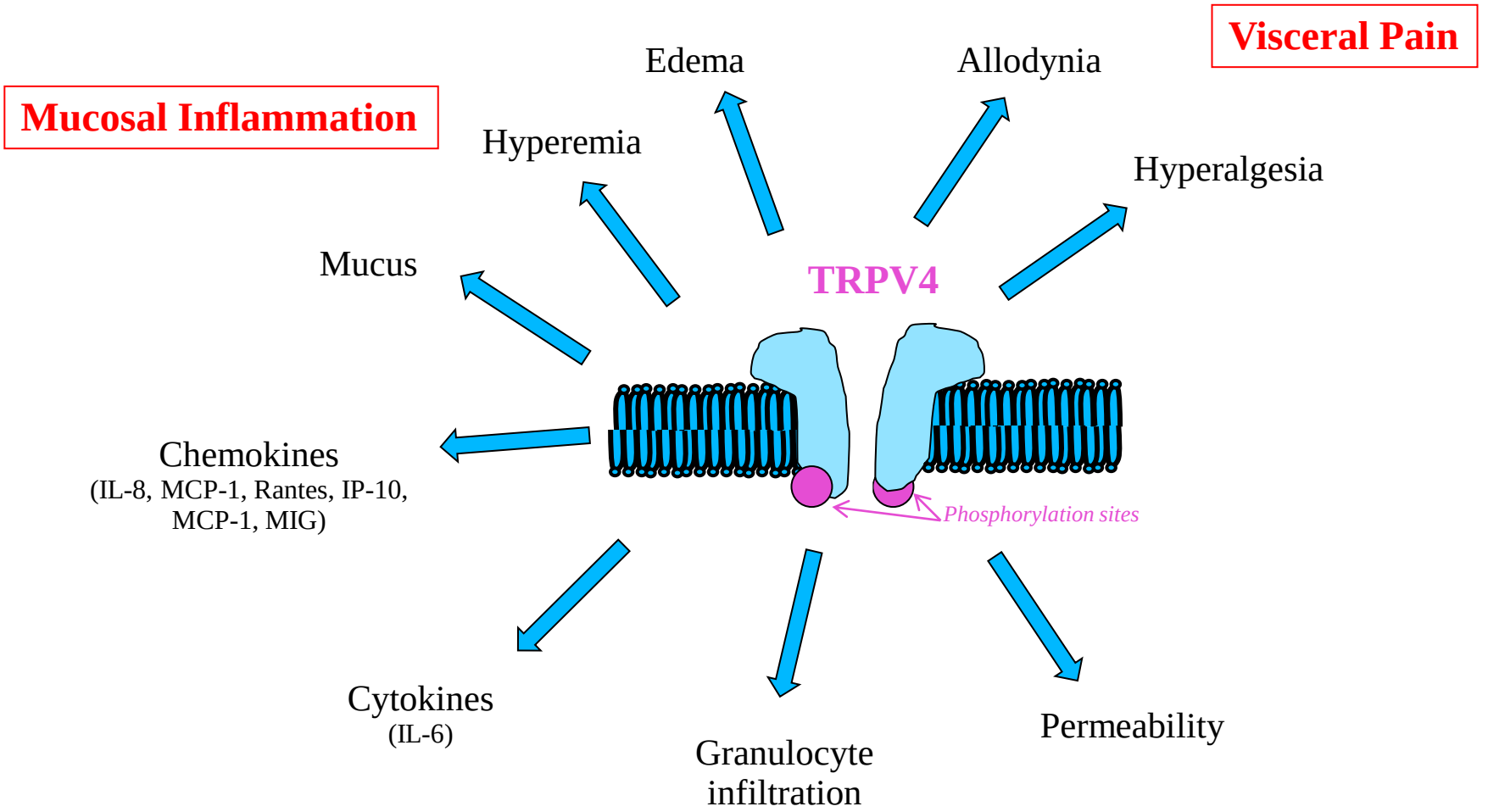
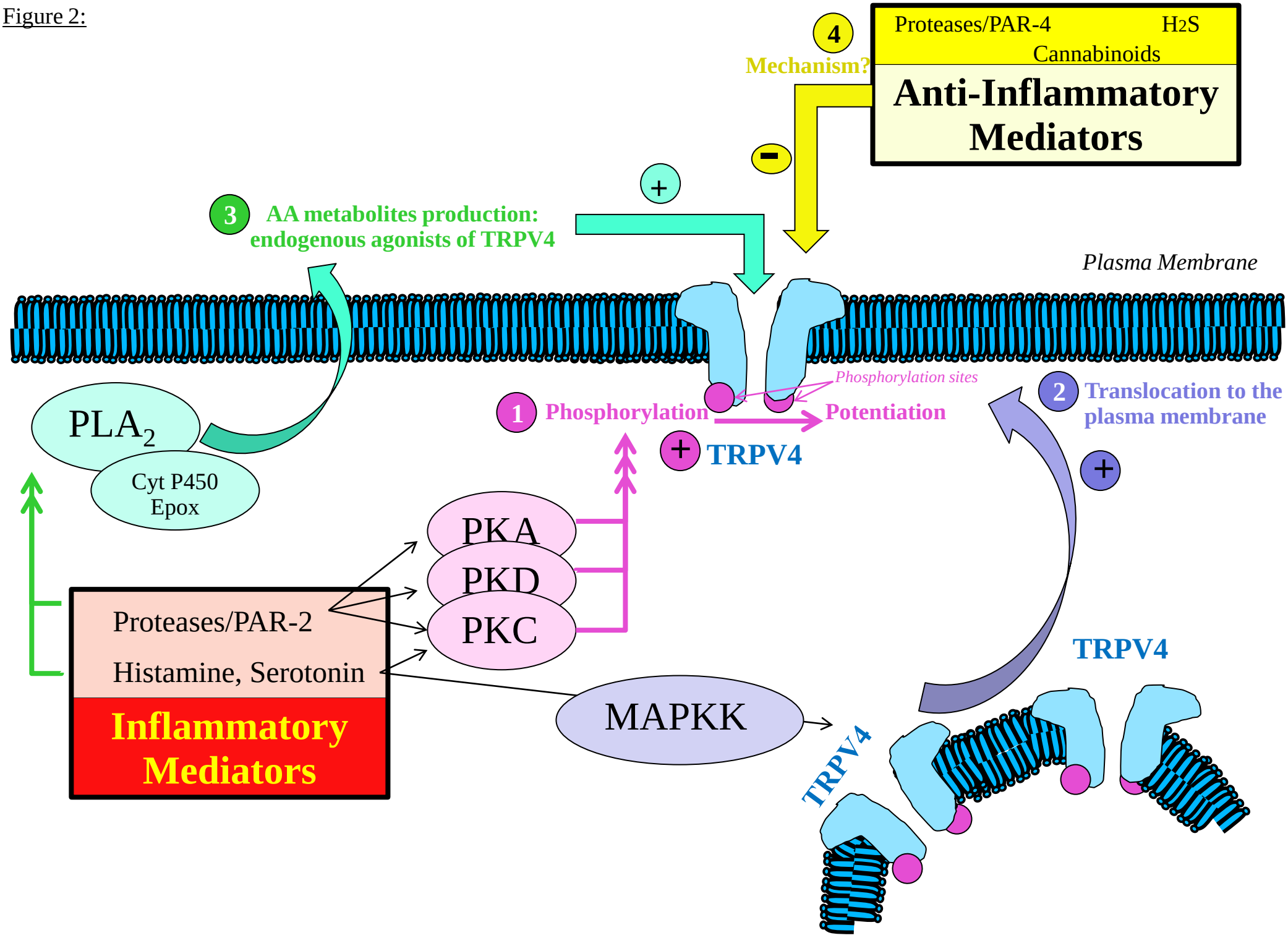


Figure 2:

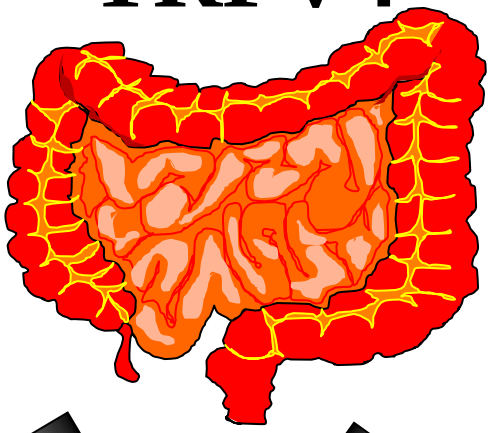

4
Mechanism?
Proteases/PAR-4
H2S
Cannabinoids
Anti-Inflammatory Mediators
+
3
AA metabolites production:
endogenous agonists of TRPV4
Plasma Membrane
Phosphorylation sites
1
Phosphorylation
Potentiation
+
TRPV4
2
Translocation to the plasma membrane
+
PLA2
Cyt P450
Epox
PKA
PKD
PKC
Proteases/PAR-2
Histamine, Serotonin
Inflammatory Mediators
MAPKK
TRPV4
TRPV4

*Graphical Abstract (for review)

Activation causes COLITIS

Inhibition protects from COLITIS

TRPV4



EXPRESSION UP-REGULATED BY INFLAMMATION

POTENTIATED BY PRO-INFLAMMATORY MEDIATORS

INHIBITED BY ANTI-INFLAMMATORY MEDIATORS