Transient Receptor Potential Vanilloid 4 Regulation of Adenosine Triphosphate Release by the Adenosine Triphosphate Transporter Vesicular Nucleotide Transporter, a Novel Therapeutic Target for Gastrointestinal Baroreception and Chronic Inflammation

Hiroshi Mihara^{a, b} Ammar Boudaka^c Makoto Tominaga^d Toshiro Sugiyama^e

^aCenter for Medical Education and Career Development, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan; ^bDepartment of Gastroenterology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan; ^cDepartment of Physiology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman; ^dDivision of Cell Signaling, National Institute for Physiological Sciences, National Institutes of Natural Sciences, Okazaki, Japan; ^eResearch Division of Molecular Targeting Therapy and Prevention of GI Cancer, Hokkaido University, Sapporo, Japan

Keywords

Gastrointestinal epithelium · Adenosine triphosphate · Transient receptor potential vanilloid 4 · Clodronate

Abstract

Background: Transient receptor potential vanilloid 4 (TRPV4) is activated by stretch (mechanical), warm temperature, some epoxyeicosatrienoic acids, and lipopolysaccharide. TRPV4 is expressed throughout the gastrointestinal epithelia and its activation induces adenosine triphosphate (ATP) exocytosis that is involved in visceral hypersensitivity. As an ATP transporter, vesicular nucleotide transporter (VNUT) mediates ATP storage in secretory vesicles and ATP release via exocytosis upon stimulation. **Summary:** TRPV4 is sensitized under inflammatory conditions by a variety of factors, including proteases and serotonin, whereas methylation-dependent silencing of TRPV4 expression is associated with various pathophysiological conditions. Gastrointestinal epithelia also release ATP in response to hypo-osmolality or acid

through molecular mechanisms that remain unclear. These synergistically released ATP could be involved in visceral hypersensitivity. Low concentrations of the first generation bisphosphate, clodronate, were recently reported to inhibit VNUT activity and thus clodronate may be a safe and potent therapeutic option to treat visceral pain. **Key Messages:** This review focuses on: (1) ATP and TRPV4 activities in gastrointestinal epithelia; (2) factors that could modulate TRPV4 activity in gastrointestinal epithelia; and (3) the inhibition of VNUT as a potential novel therapeutic strategy for functional gastrointestinal disorders.

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Introduction

Visceral hypersensitivity to stretch (mechanical) [1], temperature [2], and acid [3] plays a role in functional gastrointestinal disorders, through molecular mechanisms that remain unclear. For example, in irritable bow-

el syndrome (IBS), repetitive rectal painful distention induces rectal hypersensitivity [1]. Thermal hypersensitivity was reported in IBS patients and is positively correlated with increased intestinal membrane permeability [2].

As a stretch-activated nonselective cation ion channel, transient receptor potential vanilloid 4 (TRPV4) can induce visceral hypersensitivity [4]. We found that TRPV4 and an adenosine triphosphate (ATP) transporter, vesicular nucleotide transporter (VNUT), are both expressed throughout gastrointestinal epithelia and that a VNUT inhibitor, the first-generation bisphosphonate clodronate, inhibits TRPV4-induced ATP release [5].

In this review, we consider: (1) ATP and TRPV4 activities in gastrointestinal epithelia; (2) possible factors that modulate TRPV4 activity in gastrointestinal epithelia; and (3) inhibition of VNUT as a novel therapeutic strategy for functional gastrointestinal disorders.

ATP and TRPV4 in Gastrointestinal Epithelia

ATP signaling plays an important role in a variety of gut activities. Purinergic receptors can be divided into the P2X family of ionotropic receptors and the P2Y family of G protein-coupled receptors, both of which are involved in visceral hypersensitivity [6]. In P2X3 knockout mice, ATP release in response to mechanical distension of the esophagus and stomach is similar to that seen for wild-type (WT) mice, whereas activation of afferent nerve fibers is attenuated relative to WT mice [7]. Meanwhile, a P2X3 agonist was shown to stimulate mechanosensitive vagal afferent nerves in mouse stomach and esophagus [8], suggesting that ATP release induced by mechanical stimuli and the P2X3 receptor play important roles in baroreception in the gut. Based on the possibility that epithelial ATP release in response to luminal distension could act on purinergic receptors in submucosal nerves to transduce mechanical signals to the CNS or induce enteric reflex, especially under inflammatory conditions [6], we focused on ATP release upon epithelial TRPV4 activation.

TRPV4 was originally identified as a hypoosmolarity-sensitive ion channel that is activated by mechanical stimuli, warm temperature, and epoxyeicosatrienoic acid, the levels of which are increased in inflammatory conditions [4]. In the upper gastrointestinal tract, TRPV4 is expressed in the extrinsic nerves as well as esophageal and gastric epithelia [9, 10]. TRPV4 activation promotes VNUT-mediated ATP release in the esophagus. Heat stimulus ($>38.5^{\circ}\text{C}$) significantly increases ATP release from WT cultured mouse esophageal keratinocytes in a manner that is dependent on TRPV4 expression [9].

TRPV4 can be activated by acidity, and enhanced writhing behavior was observed within 10 min of injection of 0.7% acetate into the abdomen of mice. These effects are suppressed in TRPV4 knockout (*Trpv4*^{-/-}) mice relative to WT mice [11]. In human, stimulation of esophageal epithelial cells by acid promotes ATP release [12], whereas TRPV4-mediated increases in $[\text{Ca}^{2+}]_i$ are suppressed by extracellular protons (pH 5.0) [13]. This discrepancy could be attributed to the ability of acidic conditions to induce acid-sensing ion channel-like currents in WT or TRPV4^{-/-} esophageal keratinocytes independent of TRPV4. Gastrointestinal epithelia cells release ATP in response to not only chemical but also acid, temperature, and hypo-osmolality (Fig. 1) [14].

The VNUT modulates the storage of ATP in secretory vesicles and ATP release from these vesicles via exocytosis. TRPV4 can induce VNUT-mediated ATP exocytosis in the human gastric epithelial cell line GES-1 and activate enteric neurons [5]. Overall, the high concentrations of arachidonic acid metabolites in tissues, high temperatures, hypo-osmolality, and acidity of GI fluid may elicit ATP release from GI epithelia that in turn overstimulates GI nerves (Fig. 2).

Duodenal and intestinal microinflammation and increased permeability are pathophysiological conditions that are associated with functional dyspepsia (FD) and IBS, respectively [15, 16]. Thermal hypersensitivity in IBS is linked to increased intestinal permeability [2]. TRPV4 activation increases epithelial permeability due to endocytosis of tight junction proteins, especially claudin4, as was shown in the mammary cell line HC11 [17], and also increases the permeability of the intestinal epithelial cell line IEC6 [18]. Acid infusion in the duodenum induces symptoms in a subset of FD patients, but not in control patients [19], whereas endogenous TRPV4 agonists such as 5,6-EET and 8,9-EET increase TRPV4-mediated epithelial increased permeability and thus might be involved in visceral hypersensitivity under microinflammation conditions. Although there are no reports of TRPV4 inhibitors being administered to humans, in mice and rats such inhibitors produced no serious adverse events [20], and thus might be valuable for controlling the gut hypersensitivity.

In mouse and human colon, TRPV4 localizes to epithelial cells and as yet unidentified cells of the submucosal and muscular layers. TRPV4 agonists can increase intracellular calcium concentrations and promote chemokine release in human colon cancer cell lines and induce colitis in mice [21]. Although TRPV4 is expressed in both the epithelium and enteric neurons in the colon and

Fig. 1. Physical stimuli-induced ATP release from gastrointestinal epithelia. **a** Release of ATP from the rat gastric epithelial cell line RGE1-01 in response to acid (pH 4.0), heat (40°C), and hypo-osmolality (half-normal saline). Acid-induced ATP release was not inhibited in the absence of extracellular Ca²⁺, the vesicle-traffic-

ing inhibitor BFA, or the VNUT inhibitor Clod. **b** Rat IEC-6 also release ATP in response to acid or hypo-osmolality ([#] $p < 0.05$ vs. Cont). ATP, adenosine triphosphate; BFA, brefeldin A; Clod, clodronate; IEC, intestinal epithelia cells; Cont, control.

TRPV4^{-/-} mice are less sensitive to colonic distension, the tissues in which the effects of TRPV4 activity predominate are unclear. In terms of visceral sensations, TRPV4-mediated ATP exocytosis via VNUT is likely involved in response to stretch or elevated temperatures. Levels of the endogenous TRPV4 agonist 5,6-EET are increased in colon tissues from IBS patients and the increase correlates with their symptoms' severity [22]. The TRPV4 inhibitor HC067047 attenuates distension-induced neural responses in isolated human colon tissue [23]. On the other hand, the VNUT inhibitors clodronate does not inhibit acid-induced ATP release in the gastric cell line RGE1-01 (Fig. 1), suggesting that acid-sensitive receptors other than TRPV4 may contribute to the majority of acid sensitivity in the gut epithelium. Additional studies are needed to elucidate the precise mechanism mediating acid-induced ATP release in the gut.

Possible TRPV4 Modulators in Gastrointestinal Epithelia

As mentioned earlier, TRPV4 is activated by hypoosmolality, mechanical stimuli, warm temperature, and epoxyeicosatrienoic acid [4]. Interestingly, lipopolysaccharides produced by commensal bacteria also directly activate TRPV4 [24]. Moreover, several factors are known to enhance TRPV4 function, including proteases, serotonin, histamine, tumor necrosis factor- α , interleukin-17 [25],

and protease-activated receptor 2. We showed that protease-activated receptor 2 expressed in the esophageal epithelium is activated by proteases such as trypsin and tryptase and that treatment of mouse esophageal keratinocytes with trypsin enhances ATP release via TRPV4 activation [26]. Serotonin and histamine increase TRPV4 expression in mouse colon neurons [27], whereas tumor necrosis factor- α and interleukin-17 can enhance TRPV4 expression in some neuronal cell types such as DRG [20, 25].

On the other hand, gastric epithelial cells infected with *Helicobacter pylori* and gastric cancer cells were shown to have suppressed TRPV4 expression due to enhanced methylation of the TRPV4 gene [10]. Emerging evidence shows that dysbiosis is involved in various diseases such as IBS that involve modulations of epithelial permeability, metabolites, and immune system function [28]. Such enhanced or suppressed function of TRPV4 can be a molecular mechanism by which visceral hypersensitivity or blunting occurs (Fig. 2).

VNUT Inhibition as a Novel Therapeutic Strategy for Functional Gastrointestinal Disorders

Blockage of nerve pathways that connect TRPV4 to ATP and ATP to a purinergic receptor should ameliorate visceral pain. Although TRPV4 inhibition had no serious systemic adverse events in mice and rats [20], in humans

Fig. 2. Proposed molecular mechanism of visceral hypersensitivity or blunting with TRPV4 enhancement or suppression. **a** Formation of major metabolites generated from the AA cascade. CYP enzymes convert arachidonic acid into EETs. Increased amounts of 5,6-EET or 8,9-EET then activate TRPV4. **b** TRPV4 expressed in gastrointestinal epithelia is activated by stretch, heat, hypo-osmolality, LPS or the endogenous activators (5,6-EET and 8,9-EET). Several factors (e.g., proteases such as trypsin and trypsinase, TNF α , serotonin, histamine, IL-17) enhance TRPV4 function. TRPV4 activation induces VNUT-mediated ATP exocytosis and

increases cellular permeability. Acid also induces ATP release via another mechanism to induce visceral hypersensitivity. **c** Methylation-silencing of TRPV4 expression decreases epithelial sensitivity to physiological stimuli resulting in diminished visceral responses. AA, arachidonic acid; CYP, Cytochrome P450; EET, epoxycosatrienoic acids; TRPV4, transient receptor potential vanilloid 4; LPS, lipopolysaccharides; VNUT, vesicular nucleotide transporter; ATP, adenosine triphosphate; TNF α , tumor necrosis factor- α ; IL-17, interleukin-17; PG, prostaglandin; LT, leukotriene; COX, cyclooxygenase; LOX, lipoxygenase.

such inhibition could promote physiological dysfunction. P2X7 receptor antagonists such as AZD-9056 were not effective in mitigating Crohn's disease symptoms. P2X3 receptor antagonists have been recommended as a possible treatment for IBS [6].

The SLC17A9 gene encodes the VNUT. VNUT knock-out mice appear to be healthy but have reduced amounts of vesicular storage and release of ATP and are resistant to acute inflammatory pain [29]. Recently, the first-generation bisphosphate clodronate was shown to specifically inhibit VNUT activity at low concentrations, suggesting that this compound could be an effective agent to treat chronic pain [30]. Indeed, intravenous injection of clodronate into mice attenuated inflammatory pain by about

40% relative to untreated animals without any observed adverse effects and had an analgesic effect that was stronger than diclofenac and comparable to tramadol [29]. The clodronate Bonefos has been used worldwide for over 30 years to treat osteoporosis, bone metastasis pain, and inflammatory bowel disease [30]. Intravenous or oral administration of clodronate has been shown to have analgesic effects and does not produce severe adverse events. Generic versions of clodronate are now available. However, there are currently no clinical trials to evaluate the efficacy of clodronates for treating functional gastrointestinal disorders, in part due to the widespread use of these compounds for non-gastrointestinal purposes and the lack of approval in Japan for their use as analgesic agents.

Conclusion

In recent years, epithelial cells were shown to function as sensory tissues via the activity of ion channel receptors such as TRPV4. Future studies will likely reveal additional pressure-, temperature-, and acid-sensing mechanisms that function in digestive tract epithelia and determine whether inhibition of VNUT via clodronate could serve as an effective treatment for visceral pain.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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