

Transient Receptor Potentials (TRPs) and Anaphylaxis

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Abstract The transient receptor potential (TRP) superfamily consists of 28 members in mammals (27 in human) that act as polymodal sensors and ion channels. They regulate cellular calcium influx, generate depolarization thereby triggering voltage dependent cellular processes, and in turn they are critical in inducing the metabolic activities of cells. It is increasingly apparent that many of the inflammatory mediators released in allergic reactions involve at least two of these ion channels, the ‘Vanilloid’ TRPV1 and the ‘Ankyrin’ TRPA1. This review mainly focuses on TRPV1 and TRPA1 and the role they have in the allergic response and how these receptors may be influenced in exercise-induced anaphylaxis. The threshold to react to an allergen for mast cells and lymphocytes can be reduced by activating the melastatin channel TRPM4. This channel is briefly discussed in the context of allergy.

Keywords Transient receptor potential · TRP · Ion channel · Anaphylaxis · Vanilloid · Ankyrin · Allergy · Histamine

Introduction: TRPV1 and TRPA1

There are excellent contemporary reviews on TRP receptors as ion channels and disease states including asthma and airway reactivity, in addition to increasing evidence of

effector mechanisms of many allergic mediators working via these channels [1–14].

TRPV1 was the first mammalian member of the TRPV subfamily to be discovered and is the most studied TRP channel. TRPV1 functions as an ion channel which is modulated by binding of diverse exogenous and endogenous compounds [15, 16]. TRPV1 is mainly expressed on sensory neurons, their afferent sensory fibers, but also in a multitude of non-neuronal cell types in kidney, pancreas, testes, uterus, spleen, stomach, small intestine, lung and liver mucous glands, keratinocytes, and epithelial cells in humans [17]. TRPV1 cloned as the receptor for capsaicin, the pungent compound of hot chili peppers. Other structurally related vanilloids, such as resiniferatoxin and olvanil, are also potent activators of the channel. TRPV1 is also activated by heat, low pH, endocannabinoids such as anandamide, and many other endogenous and exogenous compounds such as piperidine (from *Piper nigrum*), allicin, mustard oil, ethanol, divalent cations, and many more [18–20]. TRPV1 is also a voltage-gated channel activated by depolarization, and stimulation of channel activity by heat and most agonists reflects a leftward shift of the voltage-dependent activation curves [21]. TRPV1 shows remarkable desensitization after stimulation, predominantly caused by a feedback mechanism resulting from an increased intracellular Ca^{2+} after Ca^{2+} entry through the channel. Activity of TRPV1 is further modulated by a variety of intracellular molecules like calmodulin (CaM), ATP, and PIP_2 , and also by Ca^{2+} -dependent phosphorylation and dephosphorylation [19]. ATP and PIP_2 prevent desensitization and thereby support channel activation, whereas CaM has the opposite effect [22]. TRPV1 can be activated by multiple stimuli including heat, acidosis, capsaicin, allicin (in garlic), ethanol, lipoxygenase products, and intracellular lipid mediators such as anandamide [15, 23–29]. Upon activation, part of the transmembrane protein opens, creating a pore that gates calcium preferentially over sodium ions mediating an influx (inward current) into cells.

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This can result in depolarization of sensory nerves and activation of calcium-dependent cellular pathways in sensory nerves and other TRPV1-expressing cells. Activation by the above factors is dependent on sensitization or priming of the TRPV1 receptor that is controlled by intrinsic kinases, such as protein kinases A and C, calcium/calmodulin-dependent kinase II (CAMKii), tyrosine receptor kinases such as neurotrophic tyrosine kinase (TrkA), which are regulated by growth factors, such as nerve growth factor, NGF or S locus receptor kinase (Srk). All phosphorylate components of the transmembrane protein [26, 30–33]. TRPV1 is probably functionally coupled to TRPA1 via a similar PLC-dependent sensitization pathways [1].

Upon activation in sensory nerves, TRPV1 activation increases the release of neuropeptides via an active exocytosis pathway [34–36]. Sensory C fibers released expressing the TRPV1 receptor have high co-localization of the neurokinins calcitonin gene-related peptide, and substance P [37–39]. TRPV1 activation is a major pathway for the release of neuropeptides whereas the axon reflexes seem of minor significance [40, 41]. Once released, neuropeptides can alter smooth muscle tone, increase airway secretions and submucosal edema, and influence inflammatory and immune cellular responses [13, 42].

A 14–19 N-terminal ankyrin repeats-containing TRP channel has been baptized TRPA1, which is mainly found in nociceptive neurons of the dorsal root (DRG), trigeminal (TG) and nodose ganglia, but has also been found in hair and skin cells [43, 44]. It has significant co-expression with the TRPV1 on sensory neurons.

TRPA1 was initially identified as a noxious cold sensor [43]. TRPA1 functions as a promiscuous chemosensor, as it can be activated by a broad variety of natural compounds such as allyl isothiocyanate (the pungent compound in mustard oil and wasabi), allicin (from garlic), cinnamaldehyde (from cinnamon), tetra-hydrocannabinoid (from cannabis plant), and nicotine (from tobacco), and also by non-natural chemicals such as industrial isocyanates, tear gasses, hypochlorite, formalin, toluene, ozone, chlorite, and noxious components of smoke (acrolein, methacrolein, croton aldehyde) TRPA1 is also activated by endogenous substances such as 4-hydroxynonenal and 15-deoxy- $\Delta^{12,14}$ -prostaglandin J2, which are released after inflammation, oxidative stress, or tissue damage. TRPA1 is also directly activated by intracellular Ca^{2+} , intracellular Zn^{2+} , and intracellular alkalinisation [24, 45–62].

TRPA1 is an important oxidant sensor and is also activated by reactive oxygen species (ROS) in bronchopulmonary sensory neurons and is a key player in inflammatory diseases. While TRPV1 and TRPA1 are recognized as key convergence points for neurogenic inflammation and influence neuropeptide release, their critical role in manifesting allergic disease and clinical symptoms are not widely appreciated, as most of the publications and research in this area are related to basic science, molecular pain, and cellular physiology [13, 14, 61].

Histamine Works Through TRPV1 and TRPA1

Histamine is regarded as the archetypal mediator of allergic symptoms—itch in the skin, vascular leak, swelling, and smooth muscle constriction. Upon binding to the H1 receptor, a G-protein coupled receptor is activated, which in turn activates the phospholipase A2 and lipoxygenase pathways, leading to signal transduction of the TRPV1 receptor [3, 4]. The phospholipase pathway also activates kinases, which sensitize the TRPV1 receptor [63]. Phospholipase A2 also activates inositol trisphosphate, which activates calcium release from the endoplasmic reticulum, which in turn activates calmodulin-dependent kinase II which also phosphorylates and primes the TRPV1 receptor [22, 64, 65]. This permits the ion channel to open at a lower threshold to endogenous or exogenous activating compounds. Histamine-mediated itch can be blocked in animal models with TRPV1 receptor antagonism or knock-out mice [3, 4]. TRPA1 is also involved in histamine-mediated itch [5, 6]. The actions of histamine are summarized in Fig. 1.

Multiple inflammatory mediators, hormones and neurokinins activate kinases and phospholipases, which prime TRPV1 and TRPA1 to reduce their activation threshold to endogenous and exogenous stimuli. Specifically, in allergic reactions, the mast cell products including tryptase and trypsin act via the PAR2 receptors to activate protein C kinase and PKA [7–10]. Prostaglandins, leukotrienes, prolactin, acetylcholine, bradykinin, serotonin, nerve growth factor, somatostatin, substance P, tumor necrosis factor α , endothelin, adenosine triphosphate, and macrophage inflammatory protein 1 α also activate kinase systems that prime/sensitize the TRPV1 and TRPA1 [66–83]. However, TRPA1 is downstream of the Mas-related G protein-coupled receptor (Mrgpr) family which are activated by mast cell mediators and promote histamine-independent itch. Therefore, TRPA1 is involved in histamine-independent itch [6].

TRPs and Anaphylaxis

TRPV1 and TRPA1 ion channels are convergence points for mediators released in allergic reactions, and are pivotal regulatory ion channels in the major mechanisms of neurogenic inflammation. In the context of anaphylaxis, an appreciation of these pathways can help us understand the mechanisms of anaphylaxis and also threshold factors that can influence allergic reactions.

Anaphylaxis is a severe life-threatening allergic reaction [84]. The first studies on anaphylaxis by Richet, Hericourt and Portier in 1902 attributed this reaction to a loss of tolerance to Portuguese man-of-war jellyfish toxins; although it was quickly appreciated that non-toxin-related proteins could evoke anaphylaxis. As a side-point to our first reference point of scientific appreciation of the

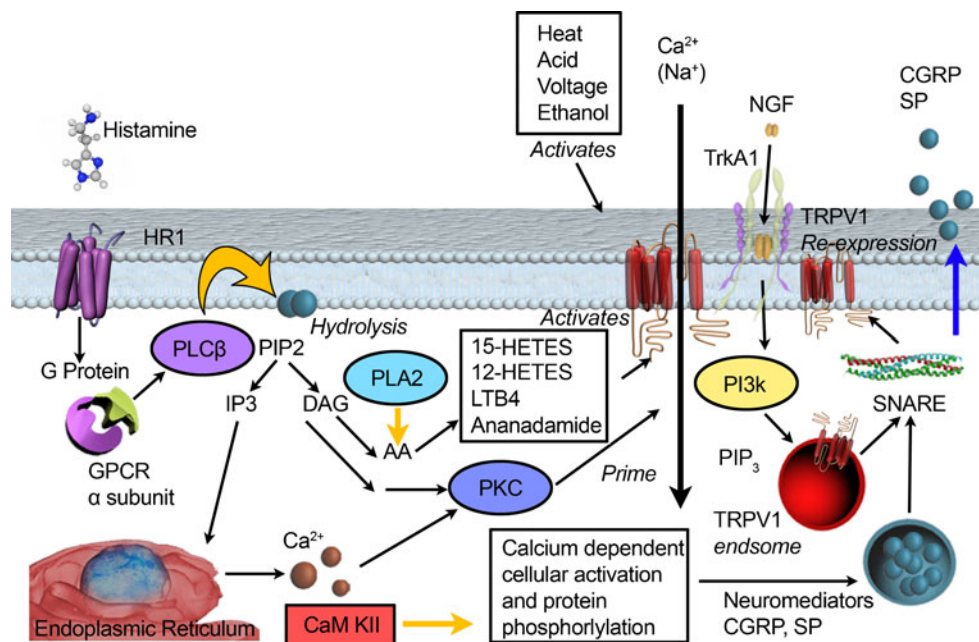


Fig. 1 Histamine binds to a G protein coupled receptor (GPCR). A specific subunit of the GPCR with bound Guanosine-5'-triphosphate then activates the phospholipase C(beta) pathway which promotes hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂), a component of the cell membrane to produce fatty acid diacylglycerol (DAG) and Inositol triphosphate (IP₃). IP₃ binds to its receptor on the endoplasmic reticulum to release intracellular calcium, which in-turn increases protein kinase C (PKC). DAG can be processed by PLA2 into arachidonic acid and lip-oxygenase products. TRPV1 may be activated by endogenous mediators including arachidonic acid metabolites hydroxy-eicosatetraenoic acids

(HETEs) and anandamide as well as exogenous activators such as acids, ethanol and high temperature. Calcium flows into the cell (preferentially to sodium), activating calcium dependent pathways. In sensory neurones this can cause itch. TRPV1 expression and reactive Peptidergic sensory neurones can release neuropeptides under TRPV1 controlled exocytosis via a SNARE (soluble N-ethylmaleimide sensitive fusion attachment protein receptor) mechanism to further increase itch as well as causing effects on mast cells and blood vessels. TRPV1 expression is controlled by stimulation by nerve growth factors (eosinophils are the most common leucocyte source) and re-expression is also SNARE-dependent

mechanisms of anaphylaxis, jellyfish toxins have highly specific binding to the TRPV1 receptor [85]. This causes neurogenic itch, pain and a dramatic “allergic” cutaneous response even without prior sensitization. A component of the historical description of the “anaphylaxis” response was direct TRPV1 activation.

Mast cells and nerves are inter-related in proximity to each other [86]. TRPV1-regulated neuromediator exocytosis evokes local tissue effects recognized in allergic reactions. The neurokinin substance P holds special interest in allergy as it has a specific receptor present on mast cells, the neurokinin 1 receptor (NK1R). The NK1R is not constitutively expressed, but it appears in the presence of the allergic cytokine interleukin 4 and also in the presence of stem cell factor [87]. Mast cell numbers are increased in number and are closer to mucosal surfaces in allergic disease. These factors could promote greater neurogenic inflammation in an atopic individual.

The severity of allergic disease (asthma, rhinitis, and atopic dermatitis) does impact as threshold factors for the severity of anaphylaxis reactions [88]. This review previously described that many allergic mediators sensitize TRP channels, making them primed and more labile to endogenous or exogenous stimuli. The eosinophil product nerve

growth factor (NGF) promotes TRPV1 expression through transcription, receptor priming, and exocytosis [89–91]. In allergic conditions such as asthma, rhinitis and atopic dermatitis, the level of TRPV1 receptors or their reactivity have been reported to be increased [12, 92, 93]. In a murine model, allergen-induced airway inflammation increased expression of TRPV1 in sensory nerves’ myelinated pulmonary afferents [94].

Alcohol is a risk factor for anaphylaxis beyond the obvious issues of recognition of risk, symptoms, and the appropriate management. Ethanol sensitizes opening of the TRPV1 channel [22], lowering the threshold to activation by other endogenous or exogenous factors and compounds (such as normally innocent temperatures or a higher pH or resting levels of endocannabinoids, arachidonic acid products, etc.). This is how ethanol causes neurogenic vasodilation [95].

Exercise-induced anaphylaxis is likely to involve both TRPV1 and TRPA1 ion channels. From a simplistic point of view, direct TRPV1 agonists heat and acidosis (a rise in core body temperature and metabolic acidosis in exercise) provide stimuli for activation of this calcium-dependent channel and neurokinin release. Acidity lowers the threshold of the ion channel opening to high temperature, capsaicin, and

endogenous activating compounds, such as anandamide or 12-HPETE, while lowering the pH has been shown to increase the ion channel response to capsaicin, indicating that these channel activating factors have cumulative effects [96–98]. Exercise is an oxidative state, with the individual consuming more oxygen and generating a range of compounds including superoxide anions, hydroxyl radicals, and hydrogen peroxide [99]. TRPA1 is the main oxidant receptor and the ion channel activates on sensing these irritant compounds [61]. Importantly, it acts as a direct sensor of oxygen and is activated during hypoxia [100]. Serotonin and bradykinin also increase with exercise [101, 102] influencing priming of TRPV1. However, bradykinin has a more direct activation effect on TRPA1 [54].

Exercise increases the production of anandamide, an endocannabinoid, which centrally provides a “reward” system for exercise [103]. From an allergic reaction point of view, anandamide is detrimental as it acts as a TRPV1 receptor agonist [104, 105]. Endorphins can also increase as part of the reward system of exercise and have been demonstrated to contribute to mast cell degranulation [106, 107]. Morphine activates the TRPV1 receptor (causing itch) via the mu opioid receptor via the PKA pathway [108].

The rise of intracellular calcium that can occur in an allergic response with activation of the TRPV1 ion channel

evokes endogenous auto-regulation processes. Calmodulin directs the TRPV1 ion channel to either a closed state or inducing a new hypo-functional state without a change in receptor numbers [64]. Calmodulin-induced desensitization can be blocked by intracellular calcium chelators [64]. Desensitization also involves calcineurin dephosphorylating two CaMKII phosphorylated residues on TRPV1 [22, 109]. ATP can block this inhibitory process, interfering with the calcineurin process, and additionally this mediator increases levels of PIP2, which is required for ion channel function [110]. Plasma levels of ATP increase with exercise [111], providing a mechanism of inhibition of the endogenous ion channel hypo-function mechanism in the presence of excessive stimulation (e.g., a severe allergic reaction). Protein kinase C (activated by bradykinin and serotonin) also inhibits TRPV1 down-regulation [112].

In summary, exercise induces a physiological state which is unfavorable to allergy. This is summarized in Fig. 2.

From a therapeutic point of view, most of the pharmacological emphasis on TRPV1 and TRPA1 has been on the treatment of pain. TRPV1 antagonists have been associated with hyperthermia [113]. Methylene blue is a guanylyl cyclase inhibitor, which should influence multiple mediators that sensitize TRPV1 and TRPA1 at the level of G-protein signaling. Methylene blue has been reported to be efficacious in treating

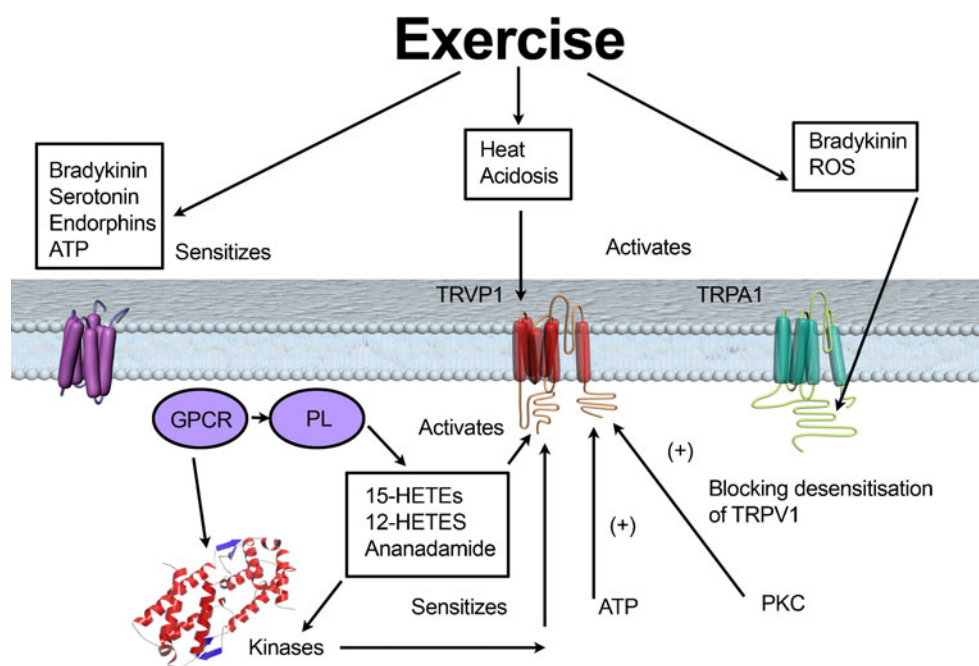


Fig. 2 Exercise induces production of mediators that bind to specific neuronal receptors and activate phospholipase (PL) A2 and C and several kinases pathways. Phospholipid activation increases arachidonic acid metabolites and the endocannabinoid anandamide that can activate the TRPV1 receptor. Bradykinin and reactive oxygen species (ROS) directly agonise TRPA1. The increase in core body temperature and metabolic acidosis of exercise are TRPV1 agonists. Exercise

induces ATP and the elevation in PKC produced by exercise induced mediators can inhibit endogenous control mechanisms for the TRPV1 ion channel. ATP binds to the site in the cytosolic ankyrin repeat domain of TRPV1 that calmodulin would normally bind to, to induce tachyphylaxis as a control mechanism to repeated ion channel stimulation. This produces an unfavorable ion channel state in the presence of allergic mediators

anaphylaxis. However, the reports have not linked its molecular mechanism of action [114, 115]. While antihistamines are useful in milder allergic conditions, their value in anaphylaxis is adjunct at best. “Mast cell stabilizing antihistamines”, such as olopatadine hydrochloride which reduces phospholipase C activity, and ketotifen and azelastine which reduce phospholipase A2 activities, could play a more important role in the treatment of severe allergic reactions [116, 117].

TRPM4 and Allergic Responses

A different TPR from another family, TRPM4, is a Ca^{2+} activated non-selective cation channel, which is not permeable to Ca^{2+} [1, 2]. In mast cells, TRPM4 depolarizes the cells in the presence of Ca^{2+} thereby reducing the inwardly driving force for Ca^{2+} entry. Its inhibition, by inducing a hyperpolarization, causes an increased Ca^{2+} dependent histamine release by increasing the driving force for Ca^{2+} entry. Thus, TRPM4 mediates a feedback mechanism. Absence of negative feedback via TRPM4-induced cell depolarization causes increased calcium influx, lowering the threshold to reactions with antigen and resulting in a more dramatic cutaneous anaphylaxis response [11]. TRPM4 is also important in the migration of mast cells and dendritic cells [118, 119]. TRPM4 also regulates calcium channels in T lymphocytes, limiting the production of NFAT-dependent interleukin (IL)-2 production in response to excessive T lymphocyte stimulation [120]. TRPM4 agonism could represent a mast cell stabilizing therapy for severe allergic reactions.

Conclusions

TRPS are key convergence points for cellular function and dysfunction in allergic states. TRPV1 expression/activity is up-regulated in allergic states. TRPA1 function is intertwined with TRPV1. These mediators are critical in the regulation of neurogenic inflammation and altering their sensitization represents a current therapeutic option, whereas specific antagonists may further contribute to improved pharmacological management of anaphylaxis and severe allergic reactions.

Disclosure Dr. Smith is the co-owner of 2 provisional patents in the treatment of molecular pain in allergy, was paid to write a review on the use of a capsaicin nasal spray in rhinitis for a pharmaceutical journal (with full disclosure), and was paid honoraria to present on the use of a capsaicin spray in treatment of nonallergic rhinitis (3 lectures in 12 months).

Dr. Nilius reported no potential conflicts of interest relevant to this article.

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