TOXICOLOGICAL HIGHLIGHT

The TRPV1 Receptor: Target of Toxicants and Therapeutics

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Understanding the structural and functional complexities of the transient receptor potential vanilloid receptor (TRPV1) is essential to the therapeutic modulation of inflammation and pain. Because of its central role in initiating inflammatory processes and integrating painful stimuli, there is an understandable interest in its pharmacological manipulation (sensitization/desensitization). The present Highlight entitled “TRPV1 antagonists elevate cell surface populations of receptor protein and exacerbate TRPV1 mediated toxicities in human lung epithelial cells” describes how exposure to various antagonists produces TRPV1 sensitization and proposes a possible mechanistic explanation to that sensitization.

Key Words: TRPV1; capsaicin receptor; neurogenic inflammation; respiratory inflammation; BEAS-2B; particulate matter.

The TRPV1 receptor is probably the best understood member of the transient receptor potential (TRP) family receptors (Reilly et al., in press; Caterina et al., 2001). Before its cloning, the transient receptor potential vanilloid receptor (TRPV1) receptor (formerly known as vanilloid receptor (VR1)), was thought to be confined to sensory C nerve cell bodies and fiber terminals. The high-affinity 3H-resiniferatoxin (RTX) radiolabel used in early studies was limited in only labeling TRPV1 expression in peripheral tissue homogenates and was not suited for cell type–specific localization. With the onset of molecular tools, the TPVR1 is now known to be broadly expressed in all “port of entry” tissues (e.g., skin, gut, airways, conjunctiva) and the various cell types lining such tissues (i.e., keratinocytes, epithelia, endothelia, etc.). PCR amplification of specific nucleotide sequences has identified TRPV1 receptors in various peripheral nonneuronal tissues of rodents and humans (e.g., kidney, lung, testis, pancreas, spleen, liver, stomach: skin, vascular smooth muscle, placenta, cornea, uterus, and bladder). TRPV1 and its splice variants are also expressed in numerous central nervous system (CNS) regions (i.e., medial preoptic and periventricular nuclei of the hypothalamus, motor neurons of the spinal cord, cerebellum, hippocampus, and frontal cortex), and CNS cells involved in inflammation and neurodegeneration (e.g., astrocytes, microglia). Fluorescent labels have identified more precise subcellular locations of TRPV1 expression on cell membranes, smooth endoplasmic reticulum, and the Golgi complex.

The TRPV1 is activated by various ligand-like agents and a plethora of seemingly unrelated stimuli such as chemical irritants, inflammatory mediators, and tissue damaging stimuli. These include capsaicinoids such as capsaicin, RTX, and olvanil; endogenous ligands such as anandamide (which also activates the cannabinoid 1 receptor); and inflammatory mediators (e.g., phorbol-12-myristate13-acetate, lipoxygenase products, leukotriene B4, phorbol-12-phenylacetate 13-acetate 20-homovanillate. TRPV1 is also activated by nonselective stimuli such as high temperature (>43°C), acidic pH (<5.3), intracellular redox states, and electrostatic charge. The precise mechanisms of receptor activation by such agents have not been fully established although such stimuli appear to alter protein conformation and stability through specific amino acid residues on the receptor, which results in ion influx and disruptions of structural gating.

TRPV1 and the vanilloid receptor-like (VRL) receptors are members of the superfamily of TRP/vanilloid receptor ion channels. Ligand binding requires a vanilloid ring structure for binding and activation of the receptor. Currently, six members of the TRP/vanilloid receptor family of ion channels (TRPV1 to TRPV6) have been identified and characterized (Montell et al., 2002). Several other TRPV1-like proteins with homologous sequences, functional similarities, and overlap in their substrate or ligand-binding and activation profiles have been identified. Each member of the TRPV class of TRP ion channels exhibit
related, but unique, ligand binding and activation profiles. However, they all share the common feature of having multimeric cation channels which are highly selective for calcium. The formation of heteromultimers among members of the TRP family receptors may increase their functional diversity. For example, multimer formation between TRPV1 and TRPV2 and extensive coexpression of the two receptors has been localized in the IV, V, and VI layer neurons of the adult rat cerebral cortex. Structurally, the TRPV1 subunits appear to have six transmembrane (TM) domains and a short pore-forming hydrophilic stretch between the fifth and sixth TM domains. Since its cloning in 1997 (Caterina et al., 1997), many of these regions and key amino acids that are involved in specific functions (multimerization, capsaicin action, proton action, heat activation, desensitization, permeability, and phosphorylation and modulation by lipids and ATP) have been identified. Manipulation of these regions and amino acids could prove useful for the development of novel anti-nociceptive or anti-inflammatory agents.

The activation of TRPV1 receptors on sensory fibers and some nonneuronal cells (e.g., respiratory epithelia) produces calcium and sodium influx and the corresponding release of tachykinin neuropeptides (substance P [SP], neurokinin A [NKA], and calcitonin-gene-related-peptide [CGRP]). Various resident immune cells (e.g., macrophages), peripheral target cells (endothelia, epithelia), and tissues (smooth muscle) respond to these neuropeptides and mediate the tissue response characteristic of neurogenic inflammation, namely redness, swelling, and pain (Richardson et al., 2002). Vasodilation, plasma extravasation, and hyperalgesia are all mediated by NKA and SP receptors located on endothelial cells of the microvasculature and smooth muscle cells. Another aspect of the neurogenic inflammatory cascade is the release by nerve and nonneuronal cells of pro-inflammatory cytokines and substances that ultimately promote and sustain the inflammatory events. Various cell types, including mast cells, epithelial cells, and immune cells, have been shown to release pro-inflammatory cytokines (e.g., IL1β, IL6, IL8, and TNFα) in response to SP, CGRP, and NKA. The sensitivity of this receptor to numerous physicochemical factors (temperature, charge, acidity) and its widespread distribution in the brain and other peripheral organ systems suggest that the TRPV1 may be key to the inflammation associated with environmental chemicals. Toxicologists have reported an initiating role for TRPV1 in mediating airway inflammation (e.g., hyperresponsiveness, asthma) caused by chemical irritants and air pollutants (e.g., particulate matter, ozone, pesticides (Veronesi et al., 2001)). Identifying this receptor as a common responder to multiple chemical toxicants could explain how diverse pollutants and inhaled substances produce the respiratory dysfunction associated with environmental contaminants.

The central role played by this receptor in the initiation and modulation of neurogenic inflammation suggests that factors which influence its functional expression or numbers could also influence the organism’s response to inflammatory xenobiotics. Genetic makeup could be one such mechanistic determinant and has been used to explain could be used to explain individual differences in respiratory toxicities and disease states etc., in respiratory toxicities and disease states associated with chemical exposure. Experimental studies on particulate matter, diesel exhaust, ozone, and nitrogen dioxide–induced airway inflammation are marked by species, strain, and genetically driven differences in the animal’s response. A linkage between the TRPV1 and the reported differences in airway inflammation associated with residual oil fly ash (ROFA) has been explored experimentally (Veronesi et al., 2000). Whole-body airway hyperresponsiveness and bronchial alveolar lavage measures, taken on ROFA-exposed BALB/c and C57 blk/6 mice showed distinct differences in their response. To determine what subcellular events might be influencing this, explants of sensory dorsal root ganglia (DRG) neurons from each mouse strain were exposed to ROFA or prototype irritants (acid pH, capsaicin), and their response recorded using single-cell calcium imaging, inflammatory cytokine release, immunohistochemical staining of TRPV1-bearing sensory neurons, and finally, by RT-PCR. Marked differences were noted between the mouse strains and related immunocytochemically and by RT-PCR to the number of these receptors in the sensitive BALB/c and nonresponsive C57blk/6 (i.e., B6) mice. Such studies pioneered the notion that variable inflammatory sensitivity to particulate matter (i.e., ROFA) observed in different mouse strains (i.e., BALB/c, B6) might relate to the quantitative differences observed in the TRPV1 receptors found on sensory neurons that innervated the nasal and upper pulmonary airway. Cellular models have corroborated these findings in more recent studies (Reilly et al., 2003) and have demonstrated that human respiratory epithelia, transfected to overexpress TRPV1, released higher levels of inflammatory cytokines and showed increased cytotoxicity in response to capsaicin and other TRPV1 agonists relative to normal respiratory epithelia. Together, these studies suggest that genetic predisposition or molecular manipulation of the number of TRPV1 receptor modifies the animal or cellular inflammatory response to air pollutants.

Because of its central role in neurogenic inflammation, TRPV1 can be envisioned as an excellent target for pharmacological intervention of pain and inflammation. The use of capsaicinoids for pain treatment has historical precedence and is still featured in numerous over-the-counter analgesic products (Robbins, 2000). Understandably, considerable effort has been made by the pharmaceutical industry to understand and clinically benefit from the intricacies of TRPV1 (de)sensitization. Chronic exposures of capsaicin, RTX, or olavin decreases neurogenic inflammation by depleting SP, down-regulating the TRPV1 receptor present on capsaicin-sensitive nerve fibers, and in extreme cases, causing degeneration of TRPV1-expressing sensory neurons. The use of RTX, olavin, and other more selective and potent vanilloids for treatment of various
maladies has gained popularity. Currently, there are several new derivatives of capsazepine under investigation for the treatment of pain and other TRPV1-mediated processes. These chemicals (e.g., olvanil) exhibit a wide range of properties that include both agonist and antagonist activity, greater selectivity for TRPV1, lower effective concentrations, lower side-effects, increased binding affinities, and increased stability.

In contrast to desensitization of the receptor, its up-regulation contributes to the development and maintenance of chronic pain states or hyperinflammatory response. For example, the up-regulation of TRPV1 in inflammatory bowel disease in functional dyspepsia and irritable bladder has identified it as an effective target of novel therapies for chronic abdominal pain. TRPV1 antagonism by selective (i.e., capsazepine, iodoresiniferatoxin) and nonselective (ruthenium red) agents can modulate pain and the inflammatory response by receptor binding and subsequent TRPV1 signaling processes. Capsaicin antagonists, including ruthenium red and capsazepine, have been shown to inhibit the activation by noxious heat of the TRPV1 receptors expressed in nonneuronal host cells and cultured DRG.

With this background, attention is turned to the manuscript featured in the present Highlight entitled “TRPV1 antagonists elevate cell surface populations of receptor protein and exacerbate TRPV1-mediated toxicities in human lung epithelial cells” (Johansen et al., 2005). The study provides compelling data on the versatility of receptor expression and suggests a novel pathway to explain the sensitization of TRPV1 receptors. In general, sensitization may be governed by a change in conformation status of the receptor or ion channel itself, rendering an enhanced activation state. Sensitization may further be governed by increased expression of functional receptors on the cell surface. Studies using calcium up-take, ³H-RTX binding, and immunoblotting have shown that treatment of TRPV1-bearing neurons with nontoxic doses of capsaicinoids and other TRPV1 ligands (RTX and olvanil) down-regulate TRPV1 receptor expression and produce cell death. In the present study, the authors show that preexposure to three different classes of receptors antagonists (i.e., LIO328, SC0030, capsazepine) enhances TRPV1 receptor-driven cellular responses such as calcium flux, inflammatory mediator induction, and cytotoxicity in normal and overexpressing human respiratory epithelial (i.e., BEAS-2B). These exposures appear to increase the cell surface populations of TRPV1 receptor protein and TRPV1-mediated inflammatory events in both normal and TRPV1-overexpressing BEAS2B cells. The authors offer data suggesting that the observed sensitization of TRPV1 receptors by antagonist pretreatment is due to the increased number of receptors expressed, resulting from the translocation of existing receptors from the endoplasmic reticulum to the cell surface. As such, these data add to our limited understanding of TRPV1 sensitization phenomena. Whether this phenomenon applies to other cell and tissues bearing TRPV1 receptors awaits future studies. In their design, some issues raised in the present study should be clarified. For example, although it is well known that allosteric factors can influence changes in threshold activation and alter Kd values, the influence of such factors on TRPV1 sensitization is unknown. It will also be important to determine if, as in the present study, different antagonists (at different concentrations and time points) influence the magnitude of sensitization differentially. Pharmacologically, these parameters should be consistent, since the concentration and duration of exposure will certainly affect the magnitude and possible pathways of receptor sensitization and desensitization. If the agonist-related sensitization of TRPV1 receptors can be reproduced in other TRPV1 bearing cell types, an exciting and novel behavior of antagonist exposure on sensitization will have been identified. Even at this initial stage of presentation, however, the data sets reported in the present study should raise a cautionary note to the future use of TRPV1 antagonists for the therapeutic treatment of respiratory inflammation.

REFERENCES


