Review

Multisteric TRPV1 nocisensor: a target for analgesics

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Cloning of the transient receptor potential vanilloid type 1 (TRPV1), the heat-gated cation channel/capsaicin receptor expressed by sensory neurons, has opened the door for development of new types of analgesics that selectively act on nociceptors. Here we summarize mutagenetic evidence for selective loss of responsiveness to vanilloids, protons, and heat stimuli to provide clues for avoiding on-target side effects of hyperthermia and burn risk. It is suggested that the complex chemoceptive thermosensor function of TRPV1 (which is modulated by depolarizing stimuli) can be attributed to multisteric gating functions. In this way, it forms the prototype of a new class of ion channels different from the canonical voltage-gated and ligand-gated ones. Several endogenous lipid ligands activate and inhibit TRPV1 and its gating initiates sensory transducer and mediator-releasing functions. Second generation TRPV1 antagonists that do not induce hyperthermia are under development, and a dermal capsaicin patch is already on the market for long-term treatment of neuropathic pain.

TRPV1 a molecular sensor on nociceptors

Nociceptors are sensory nerve endings for signaling noxious tissue-damaging stimuli which induce pain in humans. The largest group which responds to noxious heat, mechanical, and irritant chemical stimuli are thus denoted as polymodal receptors.

Identification of C-polymodal nociceptors and the selective action of capsaicin on these primary afferent neurons spurred interest in the development of a new class of analgesics that could selectively block pain signals at the level of nociceptors [1–3]. A real breakthrough occurred 15 years ago when the receptor of capsaicin (TRPV1) was cloned [4], because it revealed that TRPV1 acts as an integrator of painful stimuli [5]. This novel nonselective cation channel is gated by diverse exogenous stimuli such as noxious heat (>43 °C), protons, irritants, and also by some endogenous lipophilic compounds such as anandamide [6], products of lipoxygenases such as 12-*S*-hydroxyeicosatetraenoic acid (12-*S*-HPETE) [7], *N*-oleoyldopamine (OLDA) [8], and oxidized metabolites of linoleic acid [9] (Figure 1). In this regard, TRPV1 became the first member of a thermosensitive cation channel family. It integrates nociceptive signals expressed in the largest subset of nociceptive somatosensory neurons. Identical terms for these sensory nerve endings and their molecular transducers are alternately used and for their operational features the term 'allosteric' was adopted from ligand-gated channels which might induce false connotations (Box 1). The capsaicin receptor was renamed TRPV1 because of its structural similarity to the first member of this family described previously in the retina of the mutant *Drosophila* fruit fly [10]. Currently, 28 TRP channels have been identified in mammals (including humans), and of these 9 are responsive to temperature in different ranges [TRPV1–4, transient receptor potential ankyrin type 1 (TRPA1), transient receptor potential melastatin type 2 (TRPM2), TRPM4, TRPM5, and TRPM8] [11].

TRPV1 channels have become a promising target for high throughput screening (HTS) for analgesics that either block the function of the receptor or utilize the lasting loss of function of nociceptors which ensues after application of high doses of capsaicin.

These results kicked off enormous enthusiasm and activities in drug development, resulting in 1000 patents, but most have failed to deliver useful therapies. Currently, only the 8% capsaicin dermal patch has reached the market for treatment of nondiabetic neuropathic pain [12,13]. Among the TRPV1 antagonists, side effects such as hyperthermia and impaired noxious heat sensation (burn risk) were the main obstacles discovered in preclinical studies and clinical trials.

The aim of this review is to discuss evidence showing the uniqueness of TRPV1 – and probably other thermo-TRP channels – in their diverse operational features, which differ from the classical, more restrained proteins of voltage-gated and ligand-gated channels, as well as from the G-protein-coupled receptors (GPCRs). Recent data already indicate that these fresh considerations may form new approaches that could overcome the failures described with some TRPV1 antagonist drug candidates.

Structural biology

TRPV1 is a nonselective cation channel with six transmembrane domains, a pore loop region between the S5 and S6 segments, and as recently revealed, not three, but six ankyrin repeats at the N terminal [14] (Figure 2).

The 3D structure of the TRPV1 channel in the lipid membrane has been described by a cryo-electron microscopic technique at 19 Å resolution [15,16]. The ~150 Å high membrane protein contains a small region (60 × 60 Å) with a height of 40 Å and an intracellular large region shaped like a basket with a central cavity connected to the small region by four bridges. The largest part is ~100 Å

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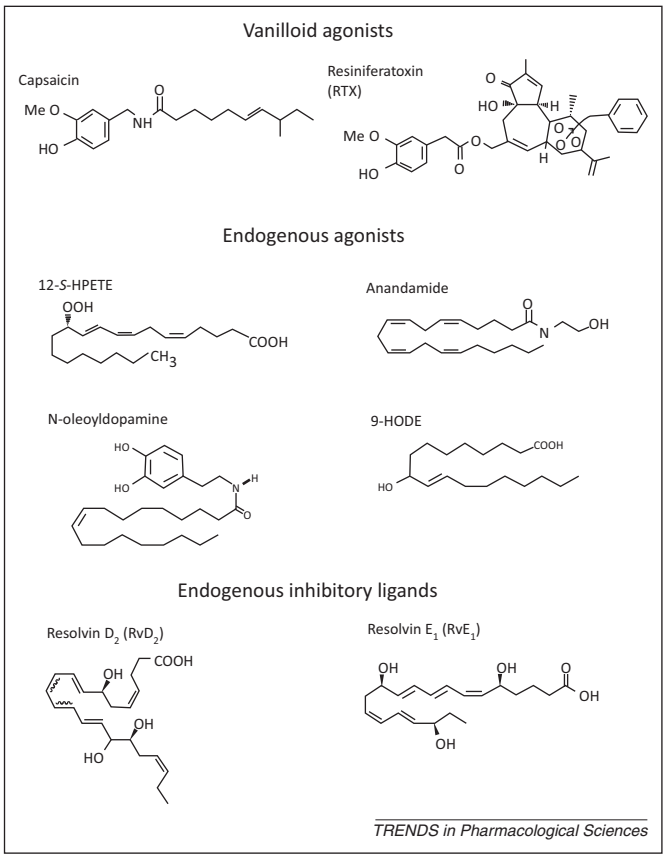

Figure 1. Chemical structures of two vanilloid exogenous agonists, capsaicin and resiniferatoxin (RTX), endogenous agonists anandamide, 12-*S*-hydro peroxyeicosatetraenoic acid (12-*S*-HPETE), *N*-oleoyldopamine, and 9-hydroxyoctadecadienoic acid (9-HODE), and endogenous inhibitory modulators resolvin D_2 (RvD_2) and resolvin E_1 (RvE_1).

wide and 110 Å high and corresponds to the cytoplasmic N and C termini. The six ankyrin repeats of the N terminus are in vertical orientation [15]. The loop region as a possible pore of the cation channel was described in the original paper on the structure of the capsaicin receptor [4], and the tetrameric – in most cases homotetrameric – structural arrangement has also been shown earlier [17] (Figures 2 and 3).

To give detailed insight into the operation of this cation channel, several mutagenic studies have been performed. Table 1 and Figure 2 (with additional studies: [14,18–20]) summarize the effects of mutagenic alterations, focusing on gating functions to various chemical and noxious heat stimuli. Similar recent overviews comparing all thermo-TRP channels reached the conclusion that these channels have unique features described as being modular proteins with allosteric gating [20] or signal integrators [5,21,22] and even polymodal nociceptors [23]. Hence, some authors classified TRPV1 as a ligand-gated channel and others as a voltage-gated channel [24,25].

Gating the channel with vanilloids

Capsaicin and resiniferatoxin (RTX) (Figure 1) contain the 4-OH, 3-OCH_3-benzyl vanilloid moiety. These agents pass through the plasma membrane to act from its intracellular side at the so-called 'vanilloid-pocket' formed by the (Ser505–Thr550) transmembrane domains of S3 and S4. Several vanilloid containing endogenous ligands such as *N*-arachidonoyl dopamine (NADA) and OLDA, as well as synthetic TRPV1 antagonists with pharmacologically distinct chemotypes, interact with this pocket. Furthermore, in the rabbit, which as a species is much less sensitive to capsaicin than rats, mice, or humans, the diminished sensitivity to capsaicin or RTX can be attributed to a single point mutation of T550I [23].

Qualitatively, capsaicin and RTX induce similar responses (e.g., excitation followed by desensitization). However, major differences were described in their potency to elicit certain responses (enhancement of $Ca^{2+}i$) and in binding to the receptor (as detected by replacement of H^3RTX) [2,26]. Before the TRPV1 channel was cloned, these mismatches provided evidence for the existence of two vanilloid receptors: R for RTX and C for capsaicin [26]. It turned out, however, that these differences were also present in TRPV1-transfected cell lines. Therefore, 10 years ago another theory was proposed [2] suggesting partly allosteric gating for these two vanilloid agonists. In the case of capsaicin, the EC_{50} in TRPV1-transfected cell lines was within the range of 6–38 nM, and on dorsal root ganglion (DRG) neurons 200–340 nM well below its K_i value for H^3RTX, which was several orders higher (600–4000 nM). The reversed order was true for RTX, where the K_d value was 18–130 pM and the EC_{50} value was 1–65 nM [2].

Mutation of M547L strongly reduced the binding and action of RTX, but increased them in the case of capsaicin [17,27]. Furthermore, mutation T550I decreased RTX binding, but not its action on $Ca^{2+}i$, and there were mutations in S3–S4 which only changed the effect of RTX, but left intact the responses to capsaicin (Figure 2, for [28–43] see Table 1).

Early structure–activity relationship (SAR) studies revealed that, for H-bonding, the 4-OH substitution at the vanilloid moiety is necessary for all actions of capsaicin [2] and also for the actions of the endogenous ligand OLDA [8,44]. In striking contrast, replacement of 4-OH with

Box 1. Polymodal receptors, allosteric, and multisteric gating

The term polymodal nociceptor (also known as polymodal receptor) was coined several decades ago to describe the unmyelinated C-afferent fibers that respond to various noxious stimuli [1]. Identified by recording from single units dissected from sensory nerves, these sensory nerve terminals respond to noxious heat, mechanical pressure, and topically applied irritants such as acid. TRPV1 and also TRPA1 are expressed in these nociceptive neurons and serve as membrane transducer proteins responsible for their polymodal nature, but designation of the term for two different entities causes confusion. The term allosteric modulation (or allosteric binding site, allosteric activation, etc.) is by definition a comparison to the primary (orthosteric) form of modulation or binding at a distinct molecular site where the natural ligand binds. Thus, allosteric modulation of TRP channels refers to actions at different molecular sites for two defined types of interventions. The term 'multisteric gating' is proposed for the TRPV1 channel (and possibly other thermo-TRP channels) on the basis of structural and functional data showing the complex conformational states for channel opening and interactions with several natural signaling processes unlike that described for canonical voltage-gated and ligand-gated channels. This view might help new approaches for drug development.