DISSECTING TRPV1
LESSONS TO BE LEARNED?

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Abbreviations: TRP, transient receptor potential; TMD, transmembrane domain; TRPV, vanilloid TRP family; TRPM, melastatin TRP family

The transient receptor potential channel TRPV1 is a polymodal nociceceptor. It is primarily expressed in dorsal root ganglia and peripheral sensory nerve endings, and to a much lesser extent, in the central nervous system. It has also been implicated in the functional properties of e.g., urinary and bronchial epithelia. TRPV1 has long been under intensive investigation by the pharmaceutical industry as a candidate drug target especially for pain conditions. This review summarizes the current knowledge of the molecular determinants of TRPV1 channel activation by heat, protons and capsaicin. Newly discovered heat and proton activation sites within the pore domain are discussed as well as potential consequences for drug discovery. Polymodal TRPV1 antagonists were found to cause hyperthermia in a species-dependent manner in-vivo, hence the discovery of euthermic compounds with an appropriate modality selectivity profile will be crucial for TRPV1's future as a drug target.

Sensory neurons from mice lacking TRPV1 are severely deficient in their responses to noxious stimuli such as capsaicin, heat or protons. These TRPV1-/- mice show normal responses to noxious mechanical stimuli but did not exhibit vanilloid-evoked pain behavior, are impaired in the detection of painful heat, and show little thermal hypersensitivity in the setting of inflammation.1 Mishra et al. have recently used a molecular genetic approach to generate mice lacking all neurons in the TRPV1 lineage and demonstrated that these animals do not respond to thermal stimuli but retain normal proprioception and mechanosensation. The mutant mice also exhibit markedly reduced ability to regulate their body temperature in response to a variety of challenges, demonstrating the importance of peripheral thermosensation in this homoeostatic process.2 For almost a decade, TRPV1 has been explored by the pharmaceutical industry as a target especially for the treatment of pain conditions. A general problem with TRPV1 as a drug target is the development of hyperthermia as an undesired on-target side-effect caused by TRPV1 antagonists tested in animal models and in clinical studies so far. Thus, for example, compound AMG517, an antagonist of all modes of TRPV1 activation, was found to elicit marked, but reportedly reversible and generally compound plasma concentration-dependent hyperthermia in Phase I clinical trials.3 Another polymodal antagonist, ABT-102, caused an increase in core body temperature in clinical trials although hyperthermic effects were effectively tolerated following twice-daily dosing for 2 days.4 Other polymodal antagonists such as BCTC, AMG9810, AMG8163, AMG3731, AMG1629 or AMG0347 all caused body core temperature increases in rats.5 As a consequence of these findings, some companies terminated their TRPV1 programs, whereas others started to explore modality-selective TRPV1 antagonists as a potential way to circumvent the development of hyperthermia in vivo.

It currently remains unclear why TRPV1 antagonism produces hyperthermia and whether it is the block of heat or proton or capsaicin activation, or combinations of these that are causative. Initially, Gavva and colleagues reported that modality-selective antagonists which are largely ineffective against proton activation, but block heat and capsaicin activation, still cause hyperthermia in a range of species and concluded that blocking capsaicin and heat activation is already sufficient to produce hyperthermia.3 In contrast, in a more recent study investigating the contribution of the different modes of TRPV1 activation to TRPV1 antagonist-induced hyperthermia, Garami and colleagues suggested that hyperthermia-free antagonists do not block the proton mode, even if they potently block the heat mode, and that decreasing the potency to block the capsaicin mode of TRPV1 activation may further decrease the potency to cause hyperthermia.6 Then, in a recent review Wong & Gavva again point to the fact that different TRPV1 antagonists which do block heat and capsaicin activation but are much less efficient in blocking proton activation still cause hyperthermia in rodents with body core temperature increases indifferent from polymodal control compounds, again questioning above mentioned hypothesis.5

In addition, the significant lack of consistency of the pharmacology of many TRPV1 antagonists across different species has been a further obstacle.6-9 AMG8562 for instance is blocking capsaicin activation in rat TRPV1 (rTRPV1) and reportedly potentiates proton activation but is comparably ineffective in blocking heat activation. In human TRPV1 (hTRPV1) however,
AMG8562 has been reported to block both heat and capsaicin activation while acting as a partial antagonist of proton activation.\textsuperscript{9} Another antagonist, JYL-1421, was shown to block capsaicin responses in rTRPV1 while it was largely ineffective in blocking heat and proton responses (pH 5.5). This apparent modality selectivity in rTRPV1 could however not be confirmed for human or monkey TRPV1.\textsuperscript{9,10}

*Summa summarum* it seems a rock solid link between block of certain TRPV1 activation modes and the development of hyperthermia in vivo has not yet been established. Moreover, translatability across different species is likewise uncertain.

The molecular dissection of TRPV1 activation modes on the other hand has gained momentum recently by the identification of heat (N628/N652/Y653) as well as novel proton activation/potentiation (F660) sites in the channel’s pore region.\textsuperscript{12,13}

It has long been known that TRPV1 can be activated by capsaicin, heat, protons as well as fatty acids such as arachidonic acid or fatty acid derivatives such as anandamide.\textsuperscript{14} More recently, C18 N-acylethanolamines (NAEs), omega-3 polyunsaturated fatty acids, oxidized linoleic acid metabolites such as 9- and 13-HODE and mustard oil have been found to activate TRPV1.\textsuperscript{15,19} However, information about how these different stimuli activate, potentiate and/or gate TRPV1 and which protein domains or which specific amino acids are involved in the different activation modes is only gradually accumulating. First, Jordt and colleagues described a glutamic residue (E600) on the extracellular side of TMD4 (V538) as critical for proton activation.\textsuperscript{22} Finally, Wang and colleagues identified two sites, one involving the pore helix (T633 in rTRPV1, corresponds to T634 in hTRPV1, Fig. 1A) and the other the extracellular loop between TMD2 and TMD3 (V538) as critical for proton activation.\textsuperscript{23} Subsequently, TRPV1 mutants (Y511A and S512Y in the intracellular loop between TMD2 and TMD3) were found which had lost their capsaicin sensitivity while maintaining normal heat and proton activation profiles.\textsuperscript{21} Five years later, Ryu and colleagues identified two sites, one involving the pore helix (T633 in rTRPV1, corresponds to T634 in hTRPV1, Fig. 1A) and the other the extracellular loop between TMD3 and TMD4 (V538) as critical for proton activation.\textsuperscript{23} Finally, Wang et al. reported that amino acid E536 in the extracellular loop between TMD3 and TMD4 is important for further stimulation of fully liganded TRPV1.\textsuperscript{23} The most recent additions to this list are the above mentioned triple mutant TRPV1(N628K/N652T/Y653T) which displays a selective loss of heat activation and TRPV1(F660) mutants (TMD6) showing a lack of both voltage-dependent proton activation and potentiation while activation by heat or capsaicin is preserved (Fig. 1A).\textsuperscript{12,13}

Taken together, we have learned from these studies which amino acids are specifically involved in heat, proton and capsaicin activation and it appears that activation and gating of TRPV1 at least by these three modes can be relatively well separated on the molecular level. However, it has also become clear that most of the amino acids involved in heat and proton activation are found in the pore region and that heat and proton activation sites are overlapping each other (Fig. 1A–E). This raises the question what level of modality selectivity can de facto be achieved with small molecule antagonists of TRPV1? A remarkable example in this context remains capsazepine. Capsazepine appears to be quite ineffective as a proton blocker in rTRPV1. Heat and capsaicin responses are blocked at much lower capsazepine concentrations: >40,000 nM (pH) versus 887 nM (capsaicin) and 6 nM (heat). However again, there is a significant species difference: in hTRPV1 all three modes of activation are blocked with similar potencies.\textsuperscript{7} The reason for these dramatically different selectivity profiles remains unknown.

Also, because a clear link between block of certain TRPV1 activation mode(s) and the development of hyperthermia in vivo is still missing, the question remains as to which mode(s) of activation can and which one(s) should not be blocked or only blocked at much higher compound concentrations. Besides that there is uncertainty whether and how modality selectivity will impact clinical efficacy. Finally, there is a lot of speculation about potential endogenous ligand(s) of TRPV1 such as fatty acids and fatty acid derivatives with no definitive knowledge about how and where—if directly on the molecule at all—such ligand(s) would activate TRPV1, and whether (modality-selective) antagonists block those endogenous ligand(s)’ effect(s). Finally, it will also be essential to understand how modality-selective activation, channel gating and ion permeation are physically coupled in TRP channels. Although Grandl et al. have shown that the pore domain is a structural determinant for thermosensitivity, Brauchi and colleagues have shown that the C-terminus domain confers thermosensitivity, gating kinetics and PIP2 modulation to TRPV1 channels.\textsuperscript{27} Besides these more mechanistic aspects relating to the design of modality-selective TRPV1 antagonists with no hyperthermic side effects, it is also important to be aware that other possible side effects may be an issue for the development of safe TRPV1 drugs. Garami et al. have recently described that deletion of the TRPV1 gene causes hypometabolism, age-related overweight, vasoconstriction and mainly hyperkinesis.\textsuperscript{28} Since TRPV1 at the periphery could regulate locomotor activity, the challenge in developing antagonists of TRPV1 with no side effects may become even more challenging.

It remains to be seen whether the concept of modality selectivity will prove true and whether any newly developed TRPV1 antagonists can fulfill all criteria necessary to be successful.

With respect to recently emerging evidence that other heat activated TRP cation channels such as TRPM3 are expressed in dorsal root and trigeminal ganglia and hence may be of interest as novel pain targets, it will be crucial to find out early on whether antagonism causes hyperthermia in vivo and if yes whether it can be circumvented with modality-selective compounds.\textsuperscript{29} What is encouraging in case of TRPM3 is the fact that the TRPM3 agonist pregnenolone sulphate was without effect on core body temperature, in spite of clear nociceptive behavior in TRPM3\textsuperscript{-/-} mice while capsaicin evoked hyperthermia in both genotypes, TRPM3\textsuperscript{-/-} and TRPM3\textsuperscript{+/+}.\textsuperscript{29}

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**Figure 1.** Location of highly-conserved residues in the pore domain involved in heat and proton activation of TRPV1. (A) Shown is an amino acid alignment of part of the TRPV1 pore domain in different TRPV1 species (h, homo sapiens; r, rattus norvegicus; o,oryctolagus cuniculus; p, pongo pygmaeus; e, equus caballus; m, mus musculus; c, cynomologus monkey). Heat-specific residues are shown in red, proton-specific residues in blue. (B) Side view of homology model structure of the pore region of one human TRPV1 subunit. The side chains of heat-specific residues are shown in red, proton-specific residues in blue. Numbers correspond to the respective positions in human TRPV1. (C) Side view of homology model structure of the pore region of all four human TRPV1 subunits. Heat-specific residues are shown in red, proton-specific residues in blue. (D) Top view of homology model structure of the pore region of all four human TRPV1 subunits. (E) Side view of the predicted surface of the TRPV1 pore region homology model. (F) Top view of the predicted surface of the TRPV1 pore region homology model. The model was generated using Modeller²⁴ based on a previously published alignment²⁵ and visualized using MoViT.²⁶
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