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Hyperthermia induced by transient receptor potential vanilloid-1 (TRPV1) antagonists in human clinical trials: Insights from mathematical modeling and meta-analysis

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ABSTRACT

Antagonists of the transient receptor potential vanilloid-1 (TRPV1) channel alter body temperature (T_b) in laboratory animals and humans: most cause hyperthermia; some produce hypothermia; and yet others have no effect. TRPV1 can be activated by capsaicin (CAP), protons (low pH), and heat. First-generation (polymodal) TRPV1 antagonists potently block all three TRPV1 activation modes. Second-generation (mode-selective) TRPV1 antagonists potently block channel activation by CAP, but exert different effects (*e.g.*, potentiation, no effect, or low-potency inhibition) in the proton mode, heat mode, or both. Based on our earlier studies in rats, only one mode of TRPV1 activation – by protons – is involved in thermoregulatory responses to TRPV1 antagonists. In rats, compounds that potently block, potentiate, or have no effect on proton activation cause hyperthermia, hypothermia, or no effect on T_b, respectively. A T_b response occurs when a TRPV1 antagonist blocks (in case of hyperthermia) or potentiates (hypothermia) the tonic TRPV1 activation by protons somewhere in the trunk, perhaps in muscles, and – *via* the acido-antithermogenic and acido-antivasoconstrictor reflexes – modulates thermogenesis and skin vasoconstriction. In this work, we used a mathematical model to analyze T_b data from human clinical trials of TRPV1 antagonists. The analysis suggests that, in humans, the hyperthermic effect depends on the antagonist's potency to block TRPV1 activation not only by protons, but also by heat, while the CAP activation mode is uninvolved. Whereas in rats TRPV1 drives thermoeffectors by mediating pH signals from the trunk, but not T_b signals, our analysis suggests that TRPV1 mediates both pH and thermal signals driving thermoregulation in humans. Hence, in humans (but not in rats), TRPV1 is likely to serve as a thermosensor of the thermoregulation system. We also conducted a meta-analysis of T_b data from human trials and found that polymodal TRPV1 antagonists (ABT-102, AZD1386, and V116517) increase T_b, whereas the mode-selective blocker NEO6860 does not. Several strategies of harnessing the thermoregulatory effects of TRPV1 antagonists in humans are discussed.

Abbreviations: A, ankyrin (as in TRPA1); CAP, capsaicin; CI(s), 95% confidence interval(s); CPZ, capsazepine; h, human (like in hTRPV1); IC_{50}, 50% inhibitory concentration of an antagonist (produces 50% of the maximum inhibitory response); i.p., intraperitoneal(ly); i.v., intravenous(ly); M, melastatin (as in TRPM8); p.o., peroral (*per os*); r, rat (like in rTRPV1); RTX, resiniferatoxin; SD, standard deviation; SDM(s), standardized difference(s) in means; T_a, ambient temperature; T_b(s), body temperature(s); TRP, transient receptor potential (channel); V, vanilloid (as in TRPV1).

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Contents

1. Introduction

Since it was first cloned by Caterina et al. (1997), the transient receptor potential (TRP) vanilloid-1 (V1) channel, formerly known as either the capsaicin receptor or VR1, has remained in the focus of pain research and drug development (Kaneko & Szallasi, 2014). Interestingly, the effects of pharmacological inactivation of this channel were studied since the 1950s (Jancso & Santha, 2015; Szolcsanyi, 2015). At that time, high doses of capsaicin (CAP), a pungent constituent of chili peppers (genus *Capsicum*), were shown to desensitize a subset of sensory nerves with consequent effects on many physiological functions. CAP is a TRPV1 agonist, and the term desensitization refers to the state of a decreased neuronal sensitivity to stimuli that normally activate TRPV1-expressing neurons, *e.g.*, noxious heat (for review, see Holzer, 1991; Szallasi & Blumberg, 1999). Studies of the desensitizing effects of CAP and other vanilloids, *e.g.*, resiniferatoxin [(RTX), an ultrapotent TRPV1 agonist naturally found in plants of the genus *Euphorbia* (Szallasi & Blumberg, 1989)], paved the way for using TRPV1 agonists to treat pain (Craft & Porreca, 1992; Szallasi & Blumberg, 1999). For example, epidermal Qutenza (CAP-containing patch) was developed for treating neuropathic pain, while intrathecal RTX has been proposed for treating pain in some forms of cancer (Chung & Campbell, 2016; Moran & Szallasi, 2018). In the late 1990s, while continuing to work on analgesic treatments based on the desensitizing property of TRPV1 agonists, multiple pharmaceutical companies had started developing TRPV1 antagonists (Holzer, 2008; Kyle & Tafesse, 2006; Lee et al., 2015). (Throughout this review, we use the terms "antagonist" and "blocker" interchangeably.) Highly potent and selective TRPV1 antagonists were, and perhaps still are, hoped to usher in a new generation of non-opioid analgesics. However, during the *in-vivo* testing of TRPV1 antagonists, adverse effects on body temperature (T_b), primarily hyperthermia, were repeatedly observed in animal studies and human clinical trials alike (*vide infra*). This review examines the thermal effects of TRPV1 antagonists and reports the results of a mathematical-modeling analysis and meta-analysis of T_b data from human trials.

In addition to vanilloids, many other stimuli are known to activate (open) the TRPV1 channel (Jordt, Tominaga, & Julius, 2000). Traditionally, when studying the TRPV1-antagonizing property of compounds, pharmaceutical companies use the following stimuli to activate this channel: CAP, low extracellular pH (< 6), and sometimes heat (> 42°C) (Fig. 1). Hence, in this review, we discuss three modes of TRPV1 activation: CAP, proton, and heat, respectively. It is now known that TRPV1 antagonists can affect these three modes differentially (for review, see Blumberg, Pearce, & Lee, 2011; Romanovsky et al., 2009). For example, a compound can potently block TRPV1 activation by CAP, but potentiate TRPV1 activation by protons (Garami, Pakai, et al., 2018; Lehto et al., 2008). In the present work, we attempted to determine the activation-mode profiles of TRPV1 antagonists that induce hyperthermia, have no effect on T_b, or even cause hypothermia in humans. Based on the results of our analyses, presented herein, we examine the strategies of minimizing the thermoregulatory effects of TRPV1 antagonists, but also of using these effects for therapeutic purposes, in humans.

2. The hyperthermic effect of TRPV1 antagonists in laboratory animals

2.1. Phenomenology

Upon systemic administration, whether intravenous (i.v.), intraperitoneal (i.p.), or peroral (p.o.), many TRPV1 antagonists cause hyperthermia in a variety of laboratory animal species, including the mouse, rat, guinea pig, dog, and cynomolgus monkey (*Macaca fascicularis*) (Fig. 2). This hyperthermia appears to be independent of chemical structure. It has been shown to develop in response to small-molecule TRPV1 antagonists belonging to different chemotypes, *viz.*, cinnamides (AMG0347 and AMG9810), pyrimidines (AMG 517), ureas (JYL 1421 and A-425619), and piperazines (BCTC), thus suggesting an on-target action (for review, see Romanovsky et al., 2009). Indeed, the on-target nature of the hyperthermic effect of TRPV1 antagonists was determined definitively, when Steiner et al. (2007) and Garami et al. (2011, 2010) showed that TRPV1 knockout mice did not increase T_b in response to either AMG0347 or AMG 517, whereas wild-type mice responded to either compound with hyperthermia.

However, not all TRPV1 antagonists are made equal as far as their ability to cause hyperthermia. While most TRPV1 antagonists produce the hyperthermic response at systemic doses in the mg/kg range (Gavva, Bannon, Surapaneni, et al., 2007; Swanson et al., 2005), others (*e.g.*, AMG0347) are effective already at 10 μg/kg (Gavva et al., 2008; Steiner et al., 2007), and yet others (*e.g.*, AS1928370) do not seem to affect T_b at all (Watabiki, Kiso, Kuramochi, et al., 2011). Some TRPV1 antagonists have been shown to be hyperthermic in several mammalian species, whereas others cause hyperthermia only in particular species. For example, AMG0347 and AMG 517 increase T_b in both rats and mice (Garami et al., 2010; Steiner et al., 2007), whereas capsazepine (CPZ) increases T_b in guinea pigs but has no thermal effect in rats (Garami et al., 2010). Intriguingly, some TRPV1 antagonists (*e.g.*, A-1165901, A-425619, AMG7905, and AMG8562) cause hypothermia instead of hyperthermia (Garami, Pakai, et al., 2018; Lehto et al., 2008; Mills et al., 2008). And yet other compounds appear to affect T_b regulation in a species-specific fashion, *e.g.*, JYL1421 was shown to cause hyperthermia in dogs and cynomolgus monkeys [Fig. 2D and E; also see Gavva, Bannon, Surapaneni et al. (2007)] but hypothermia in rats [Fig. 3D; also see Garami et al. (2010)]. Similar to the hyperthermic effect, the hypothermic effect of TRPV1 antagonists is also independent of the chemotype, as hypothermia occurs in response to small molecules with diverse chemical structures, *e.g.*, A-1165901, AMG7905, JYL1421 (Fig. 3), or AbbVie's Compound 3 (Gomtsyan et al., 2015). Two polypeptide TRPV1 antagonists, APHC1 and APHC3, have also been reported to cause hypothermia in rats (Andreev et al., 2013). In agreement with this, the hypothermic effect of TRPV1 antagonists is absent in TRPV1 knockout mice (Garami, Pakai, et al., 2018). Hence, both the hyper- and hypothermic effects of TRPV1 antagonists occur by acting on the same receptor, TRPV1, which is a highly unusual scenario. When a compound causes two opposite responses, these responses are typically mediated by different receptors (Garami, Pakai, et al., 2018). This paradox of a dual thermoregulatory action mediated by

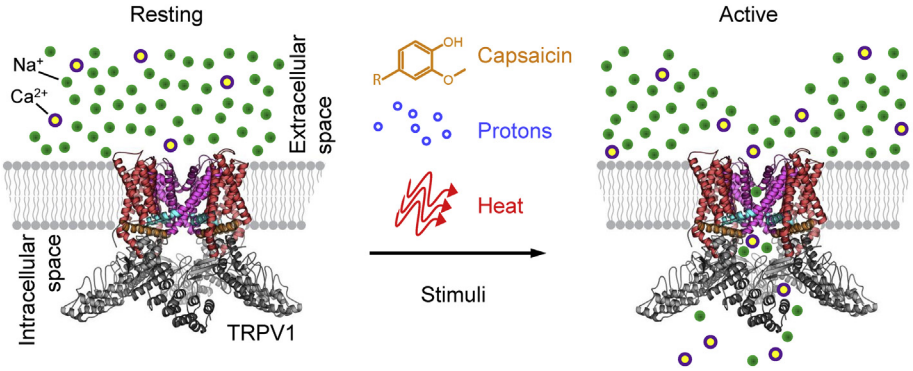


Fig. 1. TRPV1 activation: a schematic. Upon stimulation by CAP, protons, or heat, the TRPV1 channel opens, and Na^+ and Ca^{2+} cations from the extracellular space enter the cell.

the same receptor is just the tip of the iceberg – the thermoregulatory effects of TRPV1 antagonists have several other surprising physiological features.

2.2. Physiological mechanisms

Those TRPV1 antagonists that induce hyperthermia do so (at least in rats) by recruiting autonomic cold-defense effectors, *i.e.*, triggering tail-skin vasoconstriction and activating nonshivering thermogenesis in brown adipose tissue (Garami et al., 2010; Steiner et al., 2007). The same thermoeffectors – but working in reverse – bring about the hypothermic response to TRPV1 antagonists, when it occurs. In the latter case, tail-skin vasoconstriction is replaced by vasodilation, while thermogenesis is suppressed (Garami, Pakai, et al., 2018). Because the TRPV1 channel is highly sensitive to temperature (for review, see Zheng & Wen, 2019), it is often assumed that temperature signals transmitted by TRPV1 drive effector responses of the thermoregulation system, or, in other words, that the TRPV1 channel serves as a thermosensor for the thermoregulation system. Accordingly, it is further assumed that the thermoregulatory effects of TRPV1 antagonists are due to the blockade of the channel's thermosensory function (McGaraughty et al., 2009; Seebacher et al., 2010; Szolcsanyi, 2015; Vriens, Nilius, & Voets, 2014). This, however, appeared not to be the case (Romanovsky et al., 2009).

If the activity of thermoeffectors (and, consequently, the deep T_b) were to depend on TRPV1-mediated thermal signals, the magnitude of the hyperthermic effect of TRPV1 antagonists would depend on tissue temperatures in different areas of the body – deep (if the sensors are located inside the body), superficial (if the sensors are in the skin), or both. High T_bs represent strong warmth signals (that inhibit cold defenses, activate heat defenses, and – through a negative feedback loop – suppress T_b); under such conditions, TRPV1 antagonists would remove this T_b suppression and be expected to bring about a strong hyperthermic effect. Low T_bs are equivalent to the lack of warmth signals affecting thermoregulation; under such conditions, the removal of the nonexistent warmth signals by TRPV1 antagonists would be expected

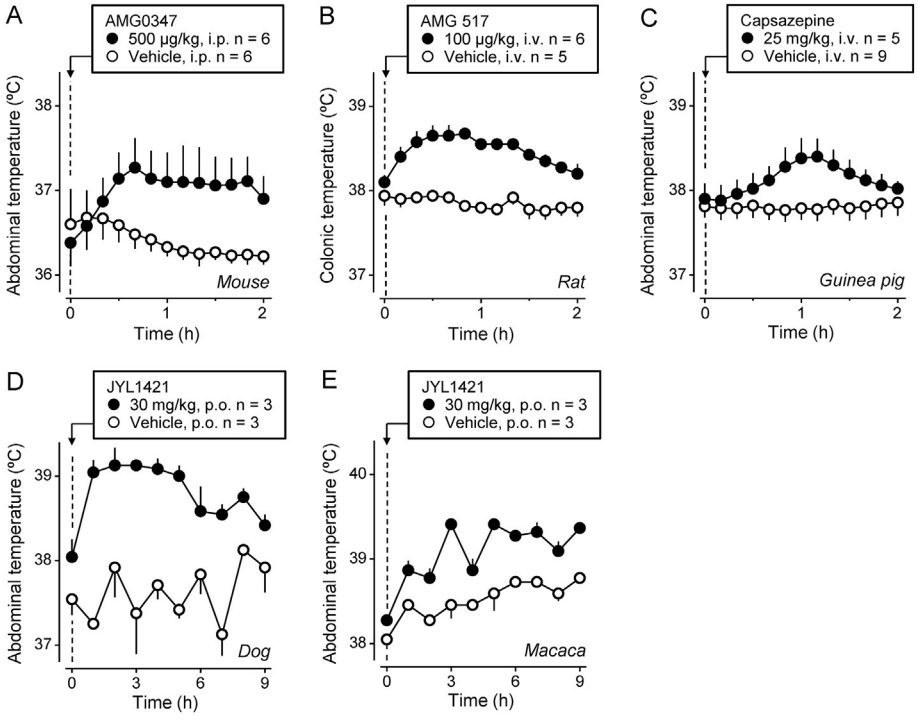


Fig. 2. Hyperthermic responses to TRPV1 antagonists in laboratory mammals. A) Effect of AMG0347 (500 µg/kg, i.p.) or its vehicle on abdominal temperature in mice at a neutral ambient temperature (T_a) of 31°C. B) Effect of AMG 517 (100 µg/kg, i.v.) or its vehicle on colonic temperature in rats at a neutral T_a of 26°C. C) Effect of capsazepine (CPZ; 25 mg/kg, i.v.) or its vehicle on abdominal temperature of guinea pigs at a neutral T_a of 27°C. D) Effect of JYL1421 (30 mg/kg, p.o.) or its vehicle on T_b (location not specified) in dogs at room temperature. E) Effect of JYL1421 (30 mg/kg, p.o.) or its vehicle on T_b (location not specified) in cynomolgus monkeys at room temperature. Modified from Steiner et al. (2007) (A); modified with permission from Gavva et al. (2008) (B); modified from Garami et al. (2010) (C) and Gavva, Bannon, Surapaneni, et al. (2007) (D, E).

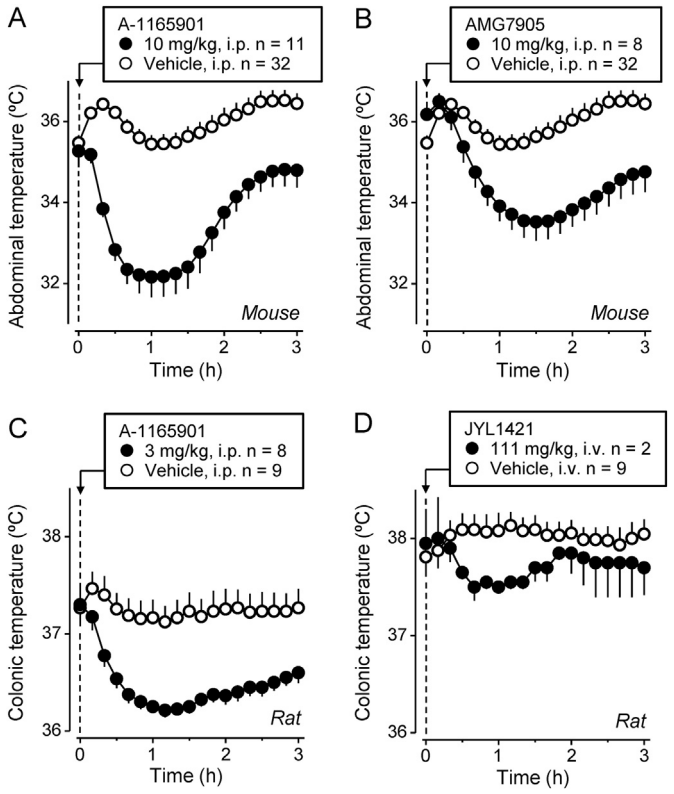


Fig. 3. Hypothermic responses to different TRPV1 antagonists in rats and mice. A) Effect of A-1165901 (10 mg/kg, i.p.) or its vehicle on abdominal temperature in mice at a subneutral T_a of 20°C. B) Effect of AMG7905 (10 mg/kg, i.p.) or its vehicle on abdominal temperature in mice at a subneutral T_a of 20°C. C) Effect of A-1165901 (3 mg/kg, i.p.) or its vehicle on colonic temperature in rats at a T_a of 26°C (the low end of the thermoneutral zone). D) Effect of JYL1421 (111 mg/kg, i.p.) or its vehicle on colonic temperature in rats at a T_a of 26°C (the low end of the thermoneutral zone). Graphs are reprinted from Garami, Pakai, et al. (2018) (A, B, C) or plotted for this work using data from Garami et al. (2010) (D).

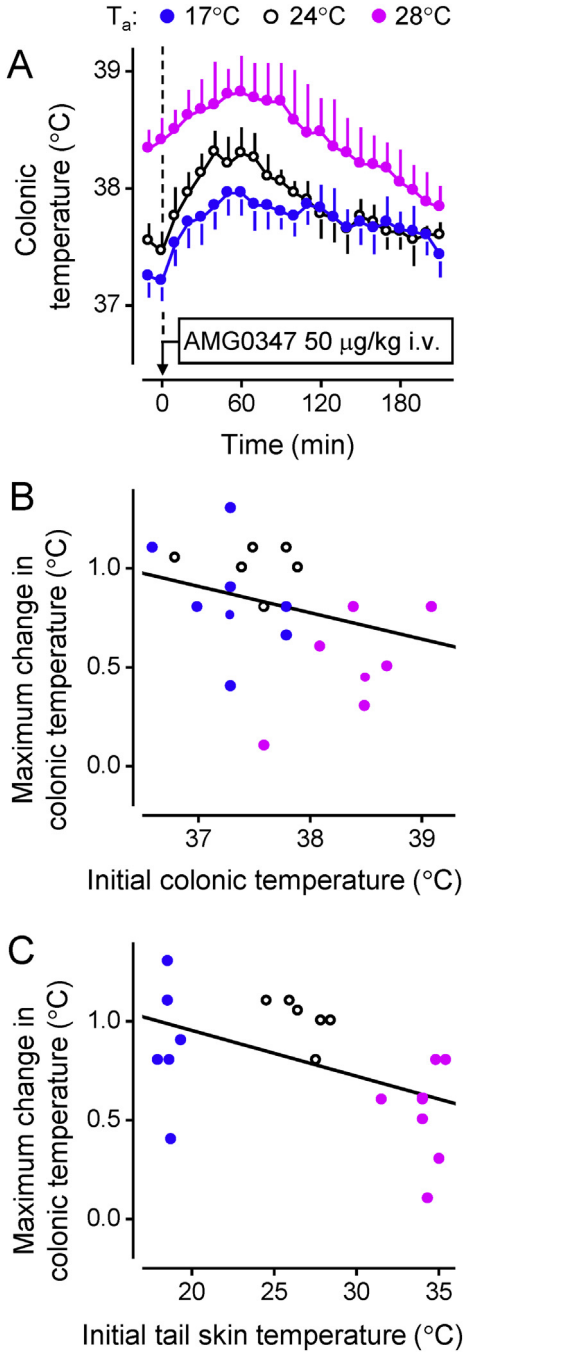


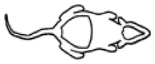
Fig. 4. In rats, the magnitude of the hyperthermic response to a TRPV1 antagonist is independent of both the initial (at the time of drug administration) colonic temperature or initial tail-skin temperature in a wide range of temperatures. A) AMG0347 (50 μg/kg, i.v.) was injected in rats at a T_a of 17, 24, or 28°C. At all T_a values tested, the drug induced hyperthermic responses of similar magnitude. No positive correlation was found when the response magnitude (the maximal increase in colonic temperature) for each rat was plotted either against the initial colonic temperature (B) or against the initial tail-skin temperature (C). Reprinted with permission from Romanovsky et al. (2009); the figure uses data from Steiner et al. (2007).

not to affect T_b at all. *Mutatis mutandis*, these assumptions were shown to be true for the cold-sensitive TRP channel melastatin-8 (TRPM8). TRPM8 antagonists cause hypothermia in rats (Almeida et al., 2012; de Oliveira et al., 2014), and the magnitude of the hypothermic response increases with a decrease in the ambient temperature (T_a) and T_bs, including skin temperatures – when the thermal (*i.e.*, cold) activation of TRPM8 is stronger, the blockade of this activation with an antagonist causes a stronger T_b response (Almeida et al., 2012). Hence, TRPM8 is a physiologically important temperature sensor that drives thermoeffector responses in the rat.

For the TRPV1 channel, the assumptions described above turned out to be incorrect, at least in the case of young male rats. Steiner et al. (2007) have analyzed whether the extent of hyperthermia depends on the initial (preinjection) temperatures (deep T_b, tail-skin temperature, and T_a) in response to an i.v. injection of the potent TRPV1 antagonist AMG0347. The authors found no positive correlation between the magnitude of the hyperthermia and any of the initial temperatures measured (Fig. 4). The lack of a positive correlation indicates that the tonic activation of TRPV1 channels, which maintains the tonic suppression of T_b, is nonthermal in nature. *A priori*, such nonthermal factors may include a low pH, inorganic cations, or endovanilloids. The fact that TRPV1 activation by heat plays no role in the thermoregulatory effects of TRPV1 antagonists (or in thermoregulation in general), at least in male rats, was confirmed in later studies [for more detail, see section 2.4 and Garami et al. (2010), Garami, Pakai, et al. (2018), Lehto et al. (2008)].

The highest levels of TRPV1 expression are found in the primary sensory neurons of dorsal-root and trigeminal ganglia, both in rodents (Jang et al., 2012; Sanchez, Krause, & Cortright, 2001) and in humans (Cortright et al., 2001). However, TRPV1 channels are widely distributed throughout the body and expressed abundantly on neural elements both within and outside of the central nervous system; they are also found in non-neural tissues (reviewed by Romanovsky et al., 2009). Where are the TRPV1 channels that mediate the T_b effects of nonthermal tonic stimuli (and, consequently, the thermoregulatory effects of TRPV1 antagonists) located? To answer this question, Steiner

Table 1
Sites of TRPV1 desensitization in RTX- or vehicle-pretreated rats

Pre-treatment	Compartment					Desensitization pattern	Desensitization extent
	Abdominal cavity	Eyes	Skin	Thoracic cavity	Brain		
Vehicle	0	0	0	0	0		None
RTX 0.2 mg/kg i.p.	X	X	X	X	X		Systemic
RTX 0.02 mg/kg i.p.	X	0	0	0	0		Localized intra-abdominal

The state of TRPV1 channels in different bodily compartments is marked as follows: X, desensitized; 0, non-desensitized. In schematics of the desensitization pattern, the desensitized compartments are shown in grey; the non-desensitized compartments are shown in white. Reprinted with permission from Romanovsky et al. (2009); the table was built based on the data reported by Steiner et al. (2007).

et al. (2007) compared the thermoregulatory effects of AMG0347 administered directly into the central nervous system (*viz.*, intracerebroventricularly or intrathecally) and systematically (i.v.). If a central administration were to cause hyperthermia at much lower (10-100 times) doses than a peripheral administration, this would indicate a central action. This, however, was not the case. The authors found that the threshold hyperthermic dose of AMG0347, when administered i.v., was ~6 μg/kg (a significant effect was observed at 10 μg/kg), but that the drug did not cause any changes in T_b when the dose of 6 μg/kg was administered centrally (either intracerebroventricularly or intrathecally). These findings allowed the authors to exclude a central origin of the hyperthermic response to AMG0347.

To obtain a more precise location of the channels responsible for the hyperthermic effect of TRPV1 antagonists, Steiner et al. (2007) administered AMG0347 i.v. in rats that had TRPV1 channels desensitized in different compartments of the body. As explained above (Introduction), desensitization means a decreased neuronal sensitivity to exogenous or endogenous vanilloids or other stimuli that normally activate TRPV1-expressing neurons, *e.g.*, noxious heat (Craft & Porreca, 1992; Szallasi & Blumberg, 1999). Steiner et al. (2007) administered repeated, escalating i.p. doses of RTX to rats to achieve different desensitization patterns (Table 1). At higher doses (~200 μg/kg), RTX impairs the function of TRPV1 channels throughout the entire body (systemic desensitization). When lower doses are used (~20 μg/kg), the desensitizing effect is limited to the abdominal cavity (localized, intra-abdominal desensitization). In the latter case, TRPV1-mediated reflexes triggered from the abdominal cavity (*e.g.*, RTX-induced writhing) are suppressed, while TRPV1 sensitivity in all other body compartments remains intact (Table 1). It was found that the hyperthermic response to either AMG0347 [Fig. 5; also see Steiner et al. (2007)] or another TRPV1 antagonist, A-889425 (McGaraughty et al., 2009), was absent in rats with localized, intra-abdominal desensitization. More recently, we have shown that the hypothermic effect of the TRPV1 antagonist A-1165901 is also abolished following the intra-abdominal TRPV1 desensitization in rats (Garami, Pakai, et al., 2018). The results in RTX-desensitized rats show that both the hyper- and hypothermic responses to TRPV1 antagonists are triggered from the abdomen, perhaps the intra-abdominal viscera or abdominal-wall muscles (for further discussion, see Garami, Pakai, et al., 2018).

To summarize this section, the following picture has emerged. TRPV1 antagonists produce their thermoregulatory effects by acting on TRPV1 channels located somewhere in the abdomen: in the abdominal viscera or abdominal-wall muscles. The abdominal TRPV1 channels are tonically activated by some nonthermal stimuli. The most common thermoregulatory effect of TRPV1 antagonists – hyperthermia – results from the blockade of this nonthermal TRPV1 activation and, consequently, from disinhibition of cold defenses. What stimuli tonically activate the abdominal TRPV1 channels under normal conditions? Why would thermoregulatory responses be triggered by nonthermal, TRPV1-mediated stimuli from the trunk? Before we answer these questions, we will take a more careful look at different ways to activate the TRPV1 channel.

2.3. Mode selectivity of TRPV1 activation and differential TRPV1 pharmacology

Rat (r) TRPV1 is the most studied TRPV1 ortholog, for which six cryo-electron microscopy structures with a resolution varying from 3.0 to 4.2 Å have been obtained, thus providing direct insight into the polymodal regulation of this channel (Cao, Liao, Cheng, & Julius, 2013; Gao, Cao, Julius, & Cheng, 2016; Liao, Cao, Julius, & Cheng, 2013). Derived from the structural biology studies, the molecular architecture of TRPV1 shows that the channel is a tetramer with six transmembrane helices (S1-S6). The transmembrane domain is further divided into two structural (sub-)domains: the voltage-sensing-like (sub-)domain (helices S1-S4) and the pore (sub-)domain (S5-S6), as illustrated in Fig. 6. The pore domain houses the upper and lower gates that open and close in response to diverse stimuli, including CAP, protons, and heat (Yang et al., 2018).

The combined data from a variety of methods paint the clearest picture of the molecular mechanisms associated with rTRPV1 activation by CAP and related vanilloid compounds (Yang & Zheng, 2017). Early comparative studies of TRPV1 orthologs with different CAP sensitivity identified the vanilloid-binding pocket, of which there are four per a

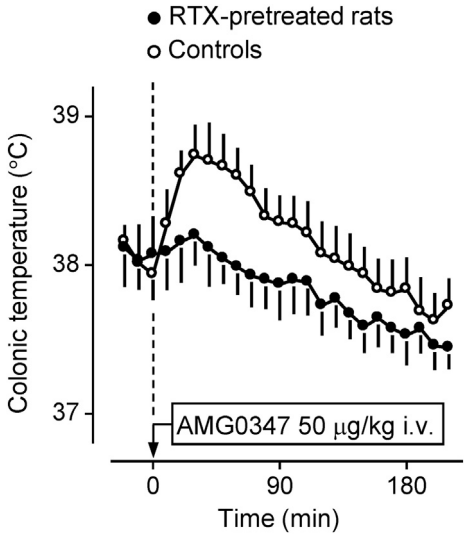


Fig. 5. The hyperthermic response to TRPV1 antagonists does not occur in rats with localized intra-abdominal TRPV1 desensitization. Shown is the abolished hyperthermic effect of AMG0347 (50 μg/kg, i.v.) in rats pretreated with RTX (20 μg/kg, i.p.). Reprinted with permission from Romanovsky et al. (2009); the figure uses data from Steiner et al. (2007).

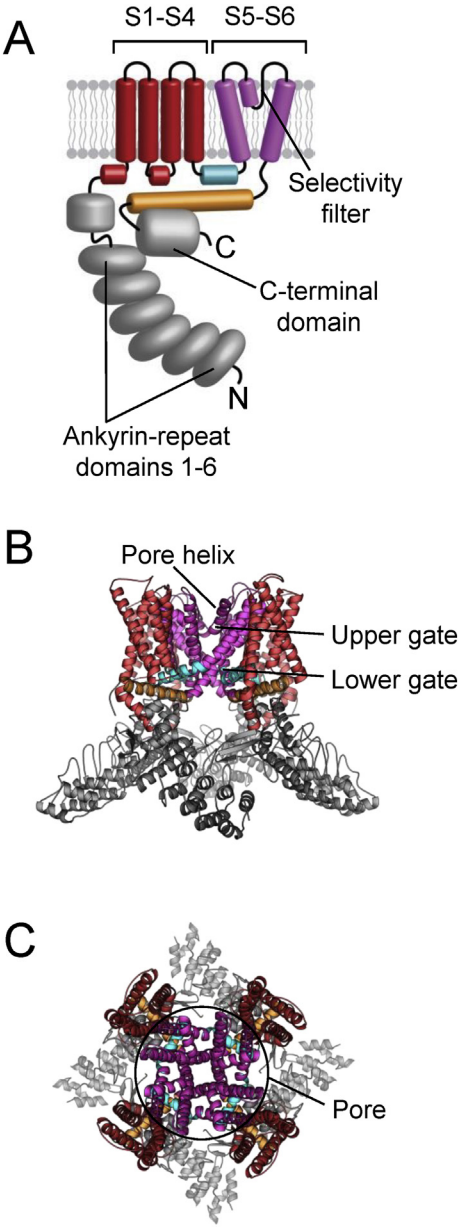


Fig. 6. Molecular architecture of TRPV1. A) A color-coded schematic of a TRPV1 monomer shows the voltage-sensing-like subdomain (S1-S4; red) and the pore subdomain (S5-S6; purple) of the transmembrane domain, as well as the S4-S5 helix linker (cyan) and the TRP helix (orange). The intracellular N- and C-termini include a series of six ankyrin-repeat domains and a C-terminal domain, respectively. The so-called selectivity filter of TRPV1 is formed from the loop that links the pore helix with the S6-helix. B) The tetrameric structure of TRPV1 (pdb ID: 3J5P) is shown with the same color-coding. The pore subdomain (purple), in its tetrameric form, includes the upper and lower gates that regulate channel activation. C) An extracellular view of the TRPV1 structure shows how the tetrameric pore subdomain forms an ion-conductive pathway (pore), which is regulated by CAP, protons, and heat.

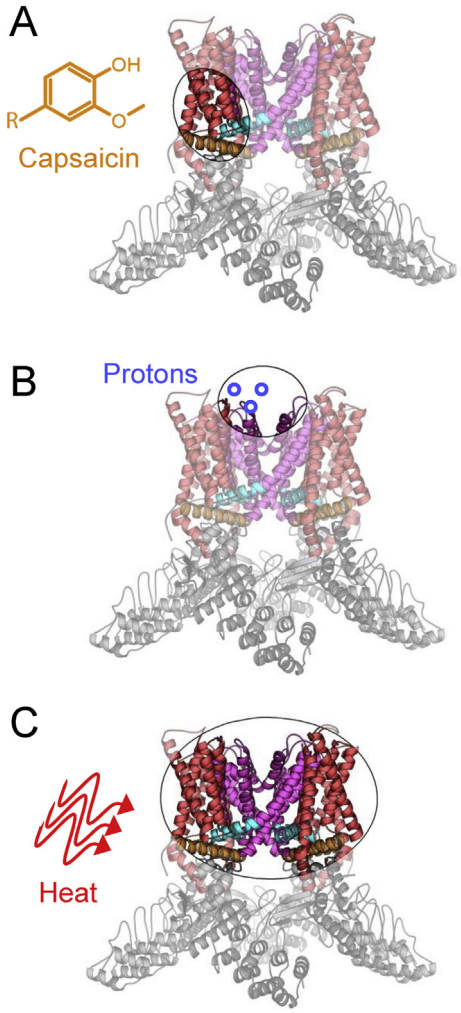


Fig. 7. Activation of TRPV1 by distinct stimuli. TRPV1 is activated by a variety of diverse stimuli, including protons, CAP, and heat, *via* spatially distinct mechanisms. A) The canonical vanilloid agonist CAP binds to the voltage-sensing-like membrane subdomain in the intracellular leaflet. B) The proton-sensing mechanism is localized primarily on the extracellular loops of the TRPV1 pore subdomain. C) The heat-activation mechanism is not well-understood. The current data suggest that the core heat-sensing architecture is harbored by the transmembrane region.

functional TRPV1 channel (Gavva et al., 2004; Jordt & Julius, 2002). The vanilloid-binding pocket (Fig. 7A) was further validated by functional, computational, and structural studies (Cao, Liao, et al., 2013; Yang et al., 2015). Activation of TRPV1 by CAP is initiated at the intracellular side of the membrane, where CAP binds to the voltage-sensing-like domain within the pocket. The vanilloid-binding pocket is energetically coupled with the pore domain, and CAP binding causes the lower gate at the S6 helix bundle crossing to open, followed by further conformational rearrangements and coupling that are propagated to the selectivity filter on the extracellular side of the membrane (*i.e.*, the upper gate), resulting in channel activation (Yang et al., 2018). While TRPV1 is the only TRPV family member that is inherently activated by vanilloids, the mechanismic understanding of TRPV1 proved to be sufficient for engineering vanilloid sensitivity into the TRPV2 and TRPV3 channels (Yang, Vu, Yarov-Yarovoy, & Zheng, 2016; Zhang et al., 2016), hence further validating the proposed model of CAP sensitivity.

Proton activation of rTRPV1 is also relatively well-studied (Boukalova, Teisinger, & Vlachova, 2013; Jordt et al., 2000). The key regions that impart proton sensitivity are located in the extracellular loops of the pore domain, across the membrane from the vanilloid-binding pocket (Fig. 7B). Canonically, two glutamate residues (E600 and E648) function as putative proton sensors, where the magnitude and range of TRPV1 pH sensitivity depend on the chemical nature of these side chains. Proton activation is initiated in the extracellular pore domain loops and then propagated to the pore helix and the upper gate (Ryu, Liu, Yao, Fu, & Qin, 2007). From there, the activation is spread to the lower gate, thus resulting in proton-dependent channel opening, *i.e.*, gating. Given the distinct spatial origins of CAP (vanilloid) and proton activation, it is interesting to note that other modes of TRPV1 regulation and activation are thought to occur extracellularly, with overlapping mechanisms to proton activation (Bohlen et al., 2010; Cao, Liao, et al., 2013; Jara-Oseguera, Bae, & Swartz, 2016). Specifically, a sodium-binding site is known to stabilize the rTRPV1 closed state in this region

(Jara-Oseguera et al., 2016), whereas a spider toxin (*i.e.*, the tarantula double-knot toxin) activates TRPV1 in the pore domain extracellular loops (Bohlen et al., 2010).

The mechanism of heat activation of rTRPV1 is still debated, as is the exact location of where heat is sensed within the channel, or even if a discreet "heat-sensor" location exists at all (Voets et al., 2004; Zheng & Wen, 2019). Indeed, virtually all areas of TRPV1 have, at one point or another, been ascribed some participation in heat activation (Hilton, Rath, Helsell, Beckstein, & Van Horn, 2015; Voets, 2014). Nonetheless, it is clear that thermosensitivity is an inherent feature of TRPV1 (Cao, Cordero-Morales, Liu, Qin, & Julius, 2013). Based on the current literature, the transmembrane region is emerging as central to thermosensitivity (Hilton, Kim, & Van Horn, 2019; also see Fig. 7C). Recent studies have shown that the pore domain of rTRPV1 is sufficient to endow a non-thermosensitive channel with heat activation, indicating that this domain is crucial for thermosensitivity (Zhang et al., 2018). There is also recent evidence that the human (h) TRPV1 voltage-sensing-like domain contributes to thermosensitivity (Kim et al., 2019). This domain undergoes a temperature-dependent conformational change that has been implicated in channel activation through the S4 helix to the pore domain, with some similarities to the mechanism of vanilloid activation. While there is strong evidence for species-specific phenotypes in TRPV1 and other TRP channels (Garcia-Avila & Islas, 2019; Hilton et al., 2015), the available data suggest that the transmembrane domain is central to heat activation, with extramembrane domains potentially modulating thermal responses. For the TRP ankyrin-1 (TRPA1) channel, a distinct thermosensitive channel, which shares the transmembrane architecture and ankyrin-repeat-based intracellular domain structures with TRPV1 (Saito & Tominaga, 2017), the temperature-sensitive region has been narrowed down to the transmembrane and C-terminal regions (Moparthi et al., 2014). More recently, temperature sensing in the TRPV3 channel has also been localized to the transmembrane domain (Singh, McGoldrick, & Sobolevsky, 2018).

Species differences, especially between rTRPV1 and hTRPV1, deserve a separate discussion. Given that the two orthologs originate from a common evolutionary ancestor and share ~85% sequence identity, as well as most general structural features, including the conserved transmembrane domain, they are expected to function similarly (Hilton et al., 2019). Indeed, the concentration of CAP that produces a half-maximal response *in vitro* is similar for the two channels (McIntyre et al., 2001), and they also have similar heat activation thresholds (McIntyre et al., 2001) and thermosensitivities (Kim et al., 2019). However, the sensitivity to protons differs between the two channels. *In vitro*, the half-maximal response occurs at the pH of ~5.8 in rTRPV1 but at the pH of ~5.5 in hTRPV1 (McIntyre et al., 2001), with the difference between the two pH values (~0.3) being very large from the physiological point of view. Hence, rTRPV1 is substantially more sensitive to protons than hTRPV1.

To summarize, it is clear that TRPV1 activation by diverse mechanisms can be spatially distinct, as evidenced by activation by CAP and protons. It is important that the spatial separation of different activation modes of TRPV1 can be exploited pharmacologically. Some TRPV1 antagonists block activation of TRPV1 in all modes (*i.e.*, by CAP, heat, and protons) with similarly high potency; these antagonists are called polymodal, mode-nonspecific (or mode-nonselective) and represent the first generation of TRPV1 antagonists. Examples of the first-generation blockers include AMG1629, AMG3731, AMG 517, AMG0347, and ABT-102 (Table 2). Yet other compounds affect TRPV1 activation in different modes differentially; they are called mode-specific (or mode-selective) and represent the second generation of TRPV1 antagonists. Second-generation compounds potently block the CAP and heat activation modes (*e.g.*, AMG2820 and AMG7988) or just solely the CAP mode (*e.g.*, SB-366791), while not affecting the remaining modes, blocking them with lower potency, or even potentiating TRPV1 activation in these modes. Examples of compounds that potentiate TRPV1 activation by protons include A-1165901, AMG8562, and JYL1421, while the TRPV1 antagonist AMG7905 potentiates TRPV1 activation by both heat and protons (Table 2). It should be noted, however, that different scientists may assign the same compound to a different generation, because the potencies of a TRPV1 antagonist in all three main activation modes are never identical, and a big difference for one author may look insignificant to another. It is also important to note that, to the best of our knowledge, all TRPV1 antagonists block TRPV1 activation by CAP with reasonable potency. This is due to the fact that pharmaceutical companies, while working on TRPV1 antagonists (at least at the early stages of their TRPV1 programs), often "discarded" any molecules that did not block TRPV1 activation by CAP – such compounds would not be considered TRPV1 antagonists. Of interest, according to R. Kapil and D. J. Kyle (personal communication), the TRPV1 program at Purdue Pharma, one of the pioneers in the field of TRPV1 antagonists, never found a molecule that blocked TRPV1 activation by protons without also blocking CAP activation.

2.4. Modeling: which modes of TRPV1 activation contribute to the hyperthermic response in rats?

We now know (Table 2) that TRPV1 antagonists can affect T_b in several ways, even in the same species (*e.g.*, the rat): many of them readily produce hyperthermia (sometimes, they are called "hyperthermic" compounds); some have no thermal effect at comparable doses ("thermally neutral" compounds); and yet others can cause hypothermia ("hypothermic" compounds). Can the ability of TRPV1 antagonists to cause different thermoregulatory responses be ascribed to their different effects on different modes of TRPV1 activation? This question was asked by scientists at Amgen (and later, among others, by scientists at AbbVie, formerly Abbott Laboratories), who synthesized a variety of compounds with differential effects on TRPV1 activation in different modes. Lehto et al. (2008) observed that, in rats, TRPV1 antagonists that potently blocked channel activation by protons (many examples are given in Section 2.3 above; also see Table 2) typically caused hyperthermia, whereas compounds that potentiated proton activation (*e.g.*, AMG8562 and AMG7905) caused hypothermia instead of hyperthermia (Table 2). However, without an advanced quantitative analysis, it is difficult to identify with certainty the relationship between the potency of a compound to block TRPV1 activation in any given mode *in vitro* and the effect of this compound on T_b *in vivo*. This uncertainty is due to, among other factors, the fact that both the thermoregulatory effect and the effect on channel activation are dose-dependent. For example, if a compound does not affect T_b, it can be inherently incapable of affecting it or, alternatively, it might have been used at a subthreshold dose for the hyperthermic effect. Furthermore, if a compound is a moderately potent blocker of TRPV1 activation in a certain mode, *e.g.*, by heat, and causes hyperthermia at moderate doses, it can be interpreted that blockers of TRPV1 activation by heat cause hyperthermia, even though the administration of a relatively low dose of this, relatively weak, blocker of TRPV1 activation by heat, could have resulted in the *in-vivo* concentrations that were insufficient to block heat activation. Not surprisingly, therefore, some studies based on comparing thermoregulatory effects of a small number of compounds administered at a couple of doses resulted in unfounded conclusions. For example, at one point, a conclusion was made that hyperthermic TRPV1 antagonists are those that block TRPV1 activation by both CAP and heat, regardless of their effect on proton activation (Gavva, Bannon, Surapaneni, et al., 2007). This conclusion was then adopted in many reviews and original-research articles (see, for example, Alawi & Keeble, 2010; Rawls & Benamar, 2011; Wong & Gavva, 2009). Yet, the subsequent research showed that this conclusion had to be modified (Garami, Pakai, et al., 2018). Clearly, a comprehensive quantitative analysis was needed.

A quantitative analysis of the contribution of the blockade of different activation modes of the TRPV1 channel to the hyperthermic response to TRPV1 antagonists was performed by our group (Garami

Table 2
TRPV1 antagonists: Their effects on deep T_b upon systemic administration in rats and their potencies at different activation modes of rat TRPV1 in vitro

Compound	Effect(s) on deep T_b		*In vitro*: IC_{50} (nM) for different activation modes			
	Effect(s)	Reference(s)	CAP (10 nM-3 μM)	pH (5.0-6.2)	Heat (45-53°C)	Reference(s)
1	↑	Gomtsyan et al., 2015			Not studied	Gomtsyan et al., 2015
74	↑	Norman et al., 2007			Not studied	Norman et al., 2007
A-1098807	↑	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
A-1098808	↑	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
A-1106625	↑	Reilly et al., 2012			Not studied	Reilly et al., 2012
A-1153818	↑	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
A-1241407	↑	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
A-889425[†]	↑	McGaraughty et al., 2009; Reilly et al., 2012		Not studied	Not studied	McGaraughty et al., 2009; Reilly et al., 2012
A-993610	↑	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
ABT-102	↑	Honore et al., 2009; Voight et al., 2014				Surowy et al., 2008
AMG1629	↑	Gavva, Bannon, Surapaneni, et al., 2007				Gavva, Bannon, Surapaneni, et al., 2007
AMG2820	↑	Gavva, Bannon, Surapaneni, et al., 2007		X		Gavva, Bannon, Surapaneni, et al., 2007
AMG3731	↑	Gavva, Bannon, Surapaneni, et al., 2007				Gavva, Bannon, Surapaneni, et al., 2007
AMG7988	↑	Gavva, Bannon, Surapaneni, et al., 2007		X		Gavva, Bannon, Surapaneni, et al., 2007
AMG8563	↑	Lehto et al., 2008		X		Lehto et al., 2008
C	↑	Gavva, Bannon, Surapaneni, et al., 2007				Gavva, Bannon, Surapaneni, et al., 2007
D	↑	Gavva, Bannon, Surapaneni, et al., 2007				Gavva, Bannon, Surapaneni, et al., 2007
E	↑	Gavva, Bannon, Surapaneni, et al., 2007				Gavva, Bannon, Surapaneni, et al., 2007

Compound	Effect(s) on deep T_b		*In vitro*: IC_{50} (nM) for different activation modes			
	Effect(s)	Reference(s)	CAP (10 nM-3 μM)	pH (5.0-6.2)	Heat (45-53°C)	Reference(s)
G	↑	Gavva, Bannon, Surapaneni, et al., 2007				Gavva, Bannon, Surapaneni, et al., 2007
H	↑	Gavva, Bannon, Surapaneni, et al., 2007				Gavva, Bannon, Surapaneni, et al., 2007
JNJ-39729209	↑	Maher et al., 2011			Not studied	Maher et al., 2011
V116517	↑	Tafesse et al., 2014			Not studied	Tafesse et al., 2014
A-425619	↔, ↑	Garami et al., 2010; Gavva, Bannon, Surapaneni, et al., 2007; Mills et al., 2008				El Kouhen et al., 2005; Gavva, Bannon, Surapaneni, et al., 2007; McDonald et al., 2008; Neelands, Jarvis, Han, Faltynek, & Surowy, 2005
AMG 517	↔, ↑	Garami et al., 2017; 2010; Gavva, Bannon, Hovland Jr., et al., 2007; 2008; Nash et al., 2012; Tamayo et al., 2008				Gavva, Bannon, Hovland Jr., et al., 2007; Tamayo et al., 2008; Wang, Katon, et al., 2007; Wang, Chakrabarti, et al., 2007
AMG0347	↔, ↑	Garami et al., 2010; Steiner et al., 2007				Steiner et al., 2007
AMG8163	↔, ↑	Garami et al., 2010; Gavva, Bannon, Hovland Jr., et al., 2007; Gavva, Bannon, Surapaneni, et al., 2007; Lehto et al., 2008				Gavva, Bannon, Surapaneni, et al., 2007; Lehto et al., 2008
BCTC	↔, ↑	Gavva, Bannon, Surapaneni, et al., 2007; Watabiki, Kiso, Kuramochi, et al., 2011				Correll, Phelps, Anthes, Umland, & Greenfeder, 2004; Gavva, Bannon, Surapaneni, et al., 2007; 2005; Kanai, Nakazato, Fujiuchi, Hara, & Imai, 2005; Papakosta et al., 2011; Valenzano et al., 2003; Watabiki, Kiso, Kuramochi, et al., 2011
BCTP	↔, ↑	Nash et al., 2012				Nash et al., 2012
JNJ-17203212	↔, ↑	Kelly et al., 2015; Swanson et al., 2005			Not studied	Swanson et al., 2005

Compound	Effect(s) on deep T_b		*In vitro*: IC_{50} (nM) for different activation modes			
	Effect(s)	Reference(s)	CAP (10 nM-3 μM)	pH (5.0-6.2)	Heat (45-53°C)	Reference(s)
JNJ-39439335	↔, ↑	Parsons et al., 2015		Not studied	Not studied	Parsons et al., 2015
JTS-653	↔, ↑	Kitagawa et al., 2012				Kitagawa et al., 2012
2	↔	Gomtsyan et al., 2015		Not studied	Not studied	Gomtsyan et al., 2015
A-1105512	↔	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
A-1165442	↔	Reilly et al., 2012; Voight et al., 2014		Not studied	Not studied	Reilly et al., 2012
A-1165746	↔	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
A-1208747	↔	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
A-1233371	↔	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
A-1233372	↔	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
A-1241797	↔	Reilly et al., 2012		Not studied	Not studied	Reilly et al., 2012
CPZ†	↔	Garami et al., 2010; Steiner, et al., 2011	X	X	X	Appendino et al., 2003; Bevan et al., 1992; Correll et al., 2004; Dickenson & Dray, 1991; Gavva et al., 2005; Jakab et al., 2005; Kanai, Hara, & Imai, 2006; Kirschstein, Greffrath, Busselberg, & Treede, 1999; Maione et al., 2007; McDonald et al., 2008; McIntyre et al., 2001; Park et al., 2003; Phillips, Reeve, Bevan, & McIntyre, 2004; Price, Patwardhan, Akopian, Hargreaves, & Flores, 2004; Rigoni et al., 2003; Savidge et al., 2002; Seabrook et al., 2002; Swanson et al., 2005; Valenzano et al., 2003
SB-366791	↔	Garami et al., 2010		X	X	Gavva et al., 2005; Varga et al., 2005
3*	↓	Gomtsyan et al., 2015		Not studied	Not studied	Gomtsyan, et al., 2015
A-1165901	↓	Garami, Pakai, et al., 2018		Potentiation	Not studied	Garami, Pakai, et al., 2018
AMG7905		Lehto et al., 2008		Potentiation	Potentiation	

Compound	Effect(s) on deep T_b		*In vitro*: IC_{50} (nM) for different activation modes			
	Effect(s)	Reference(s)	CAP (10 nM-3 μM)	pH (5.0-6.2)	Heat (45-53°C)	Reference(s)
	↓					Garami, Pakai, et al., 2018; Lehto et al., 2008
AMG8562	↓	Lehto et al., 2008		Potentiation	X	Lehto et al., 2008
I-RTX*	↓	Dogan et al., 2004			Not studied	Appendino et al., 2003; Correll et al., 2004; Johnson et al., 2006; Kanai et al., 2006; Price et al., 2004; Rigoni et al., 2003; Seabrook et al., 2002; Shimizu et al., 2005
AS1928370	↔, ↓	Watabiki, Kiso, Kuramochi, et al., 2011		X	Not studied	Watabiki, Kiso, Kuramochi, et al., 2011
JYL 1421	↔, ↓	Garami et al., 2010; Gavva, Bannon, Surapaneni, et al., 2007; Suh et al., 2003		Potentiation	X	Gavva, Bannon, Surapaneni, et al., 2007; Jakab et al., 2005; Papakosta et al., 2011; Suh et al., 2003
AMG9810	↔, ↑, ↓	Barrett, Roy, Rivard, Wilson, & Scantlebury, 2018; Garami et al., 2010; Gavva, Bannon, Surapaneni, et al., 2007; Patrone et al., 2019				Gavva, Bannon, Surapaneni, et al., 2007; Gavva et al., 2005; Norman et al., 2007

The effects on deep T_b are marked as follows: ↑, an increase; ↓, a decrease; ↔, none.
The range of IC_{50} values in the three activation modes is color-coded as follows: 1-9 nM: (CAP), (proton), (heat); 10-99 nM: (CAP), (proton), (heat); 100-999 nM: (CAP), (proton), (heat); >1,000 nM: X (any activation mode). These ranges roughly correspond to the following inhibition potencies: strong, moderate, weak, and none, respectively. When different potencies are reported for the same compound, they are shown (as different colors) within the same cell. When potentiation occurs instead of inhibition, the effect is marked as such.
Other notes: *Compound 3 and I-RTX are partial agonists (Gomtsyan et al., 2015; Shimizu et al., 2005); †CAP concentration used in the study of A-889425 by McGaraughty et al. (2009) and in the study of CPZ by Phillips, Reeve, Bevan, and McIntyre (2004) was not specified.

et al., 2010). We developed a mathematical model and used it to analyze a set of data obtained from 49 groups of rats, where each group was treated with either a distinct dose of a TRPV1 antagonist (eight antagonists were used) or vehicle. Of the antagonists studied, seven caused dose-dependent hyperthermia, whereas one (JYL1421) caused dose-dependent hypothermia. The analysis revealed that the hyperthermic response to a TRPV1 antagonist is highly sensitive to changes in the compound's *in-vitro* potency to block TRPV1 activation by protons, but is completely insensitive to the potency to block either heat or CAP activation of the channel (Fig. 8). In other words, only potent antagonists of proton activation of rTRPV1 cause hyperthermia in young male rats, and this effect does not depend on the antagonist's potency to block other modes of rTRPV1 activation. We later confirmed that those antagonists that potentiate the proton activation of TRPV1 cause hypothermia (Garami, Pakai, et al., 2018).

As explained elsewhere (Garami, Pakai, et al., 2018), the results with the CAP activation mode were initially not as clear as described in the paragraph above. In fact, the Garami et al. (2010) model produced two different outcomes, depending on whether the hypothermic antagonist JYL1421 was included in the analysis or not. The results described above and shown in Fig. 8 were obtained with a complete data set, *i.e.*, with JYL1421. If JYL1421 was excluded from the analysis, the hyperthermic response to a TRPV1 antagonist became somewhat sensitive to changes in the antagonist's potency to block CAP activation of TRPV1 – in addition to being highly sensitive to changes in the proton mode and insensitive to changes in the heat mode. At the time when the rat study (Garami et al., 2010) was conducted, we did not know whether the hyper- and hypothermic responses to TRPV1 antagonists had two distinct mechanisms (*e.g.*, one represented an off-target action) or, alternatively, whether they were brought about by the same mechanism,

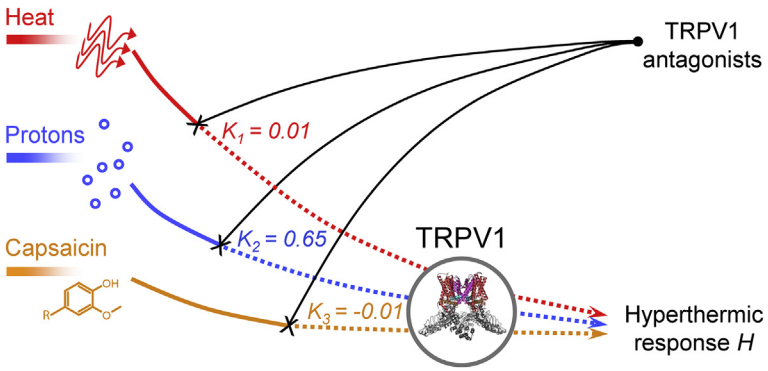


Fig. 8. Schematic presentation of the mathematical modeling results to show the contribution of different modes of TRPV1 activation to the development of TRPV1 antagonist-induced hyperthermia in rats. Signals activating TRPV1 in the heat mode (red line), proton mode (blue line), and CAP mode (orange line) are differentially blocked by TRPV1 antagonists (black lines) to cause the hyperthermic response H (see Supplementary Methods). The k_1, k_2, and k_3 values are relative sensitivities of H to the extent of TRPV1 blockade in the heat, proton, and CAP modes, respectively. Reprinted from Garami et al. (2010).

just working in reverse. In the former case, the results with JYL1421 should have been excluded from the analysis; in the latter case, they should have been included. In a more recent work (Garami, Pakai, et al., 2018), we studied the hypothermia-inducing TRPV1 antagonists A-1165901 and AMG7905 and found that the hyper- and hypothermic responses are similar from the mechanismic point of view. Both represent an on-target action (do not occur in TRPV1 knockout animals); both recruit the exact same thermoeffectors (but working in reverse); both are characterized by the same dependence of thermoeffectors on T_a; and both depend on the proton mode of TRPV1 activation (potently block *vs.* potentiate it). Hence, we concluded that TRPV1 antagonists cause hypothermia by engaging the same mechanisms as the hyperthermic TRPV1 antagonists but in reverse (Garami, Pakai, et al., 2018). Therefore, our earlier mathematical modeling study (Garami et al., 2010) should have been interpreted using a complete data set (with JYL1421), and the results obtained on the complete data set are described herein (Fig. 8).

2.5. Putting it all together: "illogical" acidification-induced anti-hyperthermic reflexes

A concept has emerged (Garami, Pakai, et al., 2018; Romanovsky et al., 2009) proposing that, at least in young male rats, TRPV1 does not serve as a thermosensor that drives thermoeffector responses – even though the thermosensing function of the channel is involved in pain in this species (Jhaveri, Elmes, Kendall, & Chapman, 2005; McGaraughty, Chu, Faltynek, & Jarvis, 2006; Vandewauw et al., 2018). Instead, TRPV1 participates in thermoregulation by sensing protons (or other stimuli that activate the channel through the same mechanism as protons). The TRPV1 channels that are tonically activated by protons and drive thermoeffectors are located somewhere in the abdominal viscera or muscles. TRPV1 antagonists affect thermoregulation, at least in young male rats, by affecting the activation of these TRPV1 channels. This picture has been drawn based on the robust experimental support (reviewed herein and elsewhere). Furthermore, as evident from the consensus paper (Garami, Pakai, et al., 2018), this view is now accepted not only by some academic scientists, but also by colleagues from Amgen and AbbVie – two companies that carried out a great amount of pioneering work with TRPV1 antagonists. And yet, this picture is rarely mentioned in the literature, while the alternative view (*i.e.*, that TRPV1 plays a thermosensory role in mammalian thermoregulation, and that TRPV1 antagonists affect T_b by blocking thermal activation of TRPV1) is widely spread. To the best of our knowledge, the thermal nature of the effector-driving TRPV1 signals – the cornerstone of this alternative view – has not been demonstrated so far, not even in a single study. Perhaps the main reason for the slow acceptance of the new reflexes, which can be called acido-antithermogenic and acido-antivasoconstrictor, is related to fact that they do not "make sense" at the first glance. Indeed, what is the biological significance of bringing the T_b down when the environment in the trunk is acidified?

Those reflexes that seem to make no sense are called "illogical" (Partridge, 1982; Romanovsky et al., 2009). The autonomic regulation, including thermoregulation, is executed by multiple, independent effector loops using both humoral and neural signals; the latter are called reflexes (Romanovsky, 2018). These reflexes are quite diverse, and only a small subset of them is active under any given set of external and internal conditions; when conditions change, a different subset of loops is recruited. We readily understand those reflexes that are vital, often engaged in everyday life of the organism, and well-studied, *e.g.*, various baroreflexes used in the cardiovascular control. Such reflexes seem "logical". Yet, there are many other reflexes that we do not understand, and some thermoregulatory reflexes that are triggered by nonthermal stimuli belong to this group. For example, skin vasoconstriction is affected by colorectal distension (Laird, Carrive, & Waite, 2006), while nonshivering thermogenesis is modulated by gastric stretching (Petervari, Garami, Pakai, & Szekely, 2005), the level of intraportal glucose (Sakaguchi & Yamazaki, 1988), and the osmolarity of the content in different parts of the gastrointestinal tract (Boschmann et al., 2007; Osaka, Kobayashi, & Inoue, 2002). All of the abovementioned reflexes seem illogical – but only until we study them, find the conditions under which they are expressed, or just start thinking about them. For example, the concentration of glucose in the portal blood, as well as gastric stretching, can be viewed as indices of energy intake, whereas nonshivering thermogenesis is a major mechanism of energy expenditure in rodents; it should not be a surprise that the latter is modulated by the former.

What could be the biological significance of the unusual TRPV1-mediated reflexes that link pH and T_b? Because polymodal TRPV1 antagonists induce robust hyperthermia in different species (Fig. 2), it is probably related to some basic physiological interactions. Initially (Steiner et al., 2007), we thought that interactions between the feeding status, gastrointestinal pH, and T_b were involved. However, in view of our recent results showing that the hyperthermic response to TRPV1 antagonists is affected neither by vagotomy (A. Garami, A. A. Steiner, and A. A. Romanovsky, unpublished observations) nor by the transection of the greater splanchnic nerves (A. Garami and A. A. Romanovsky, unpublished observations), we dismissed the visceral location of the TRPV1 channels of interest and the entire "gastrointestinal" scenario (Garami, Pakai, et al., 2018).

Instead, we propose that interactions between the acid-base homeostasis, T_b, and physical activity can be relevant. Strenuous physical activity is well-known to cause metabolic acidosis, including marked acidemia (Robergs, Ghiasvand, & Parker, 2004), and it increases deep T_b and often peripheral temperatures. Based on the tight co-expression of TRPV1 with acid-sensing ion channel-3 on

metaboreceptive afferents in muscle arterioles, it has been proposed that TRPV1 channels at this location may function as sensors for reflexes triggered by the acidic environment and elevated temperature of working muscles (Molliver et al., 2005). In those situations, when physical activity is especially strenuous (*e.g.*, when an animal is running for life from a predator), T_bs can reach extremely high values. In a study by Taylor and Lyman (1972), an abdominal temperature of > 47°C was recorded in a running gazelle. By the same token, high T_bs (whether shell or core) inhibit physical performance (Cheung & Sleivert, 2004; Nybo, Rasmussen, & Sawka, 2014; Schlader, Simmons, Stannard, & Mundel, 2011). Hence, a negative-feedback cycle is formed: an animal has to run as fast as it can to survive → T_bs increase → the capability to run decreases. Would it not be highly beneficial to counteract the development of hyperthermia by inhibiting cold-defense responses (thermoregulatory heat conservation and heat production), thus cancelling the performance-inhibiting feedback? We think it would, and the TRPV1-mediated acido-antithermogenic and acido-antivasoconstrictor reflexes discussed herein may do just that. When an animal runs, its internal environment acidifies, and the low pH, *via* TRPV1 channels (perhaps in the massive trunk muscles that are rich with slow-twitch, type-1 muscle fibers and are involved in breathing), inhibits cold defenses, thus preventing a further rise in T_b or bringing it down. This speculative line of thought has already found support in the studies showing that acute administration of CAP causes sympathetic activation and increases exercise endurance in rats and mice (Kim, Kawada, Ishihara, Inoue, & Fushiki, 1997; Oh, Oh, & Ohta, 2003). Furthermore, Luo et al. (2012) have shown that TRPV1 activation by chronic dietary CAP or transgenic TRPV1 overexpression also increases exercise endurance in mice, and that this effect of CAP does not occur in TRPV1-decifient mice. The concept presented here can be tested further by blocking TRPV1-mediated reflexes in exercising animals.

At the first glance, the proposed scenario is difficult to reconcile with the fact that lactic acid is a potent inhibitor (not activator) of TRPV1 (de la Roche et al., 2016), whereas acidosis during physical activity is accompanied by massive production of lactate (Bangsbo, Madsen, Kiens, & Richter, 1996) and, for a long time, was known as "lactic acidosis." It was believed that the increased production of lactic acid causes the release of protons and the formation of the acid salt sodium lactate, eventually exceeding the cellular buffering capacity and resulting in proton accumulation and a pH decrease. As explained by Robergs et al. (2004), the lactic acidosis concept has been disproved. While lactate production coincides with metabolic acidosis in strenuous exercise, it retards – not causes – it. In intense physical work, nonmitochondrial ATP from glycolysis is used heavily to fuel muscle contraction, thus releasing protons and causing acidosis. While extracellular lactate inhibits TRPV1, at least *in vitro* (de la Roche et al., 2016), the channel is gated open by extracellular protons (pH < 6), and milder acidosis (pH between 6 and 7) sensitizes it (reviewed by Holzer, 2009). The critical importance of TRPV1 in acid-sensing has been also demonstrated *in vivo* – using mice genetically deficient in TRPV1 (Caterina et al., 2000; Leffler, Monter, & Koltzenburg, 2006).

3. The hyperthermic effect of TRPV1 antagonists in human clinical trials

3.1. Phenomenology: effects of TRPV1 antagonists on T_b in humans

As of today, a relatively large number of TRPV1 antagonists has already been tested in humans (Table 3). Besides healthy adult volunteers, patients with a variety of conditions and symptoms were studied, often involving pain and inflammation (*e.g.*, dental or neuropathic pain, arthritis, or dermatitis), but also itching, coughing, and chronic pulmonary obstruction. As part of safety assessment, deep T_b was measured in several trials, and it is now well-known that many TRPV1 antagonists had adverse effects on T_b in humans (Gavva et al., 2008; Krarup et al., 2011; Lee et al., 2017; Manitpisitkul et al., 2015; Rowbotham et al., 2011).

As in laboratory animals (see 2.1), the thermoregulatory effects of TRPV1 antagonists in humans are diverse (Fig. 9). In one of the first human trials, AMG 517 caused marked hyperthermia with deep T_b exceeding 40°C (Gavva et al., 2008), which led to a premature termination of the trial. A dose-dependent (and, at higher doses, pronounced: 0.8-1.4°C) T_b rise was also observed in human trials with ABT-102 and V116517 (Fig. 9A and 9B, respectively), as well as with XEN-D0501 (Round, Priestley, & Robinson, 2011), while AZD1386 caused a milder (0.4°C) elevation in T_b (Fig. 9C). Another TRPV1 antagonist, SB-705498, had no thermal effect in humans, even at doses as high as 600 mg p.o. (Fig. 9D), whereas the mode-selective antagonist NEO6860 seemed to cause a small (0.2°C) decrease in deep T_b (Fig. 9E). As in laboratory animals, the thermal effects of TRPV1 antagonists in humans show no obvious association with the antagonist's chemical structure, thus suggesting an on-target action.

Because TRPV1 remains a promising therapeutic target in many human diseases and conditions (Brito, Sheth, Mukherjea, Rybak, & Ramkumar, 2014; Feketa & Marrelli, 2015; Gram, Holst, & Szallasi, 2017; Nilius & Appendino, 2013), it is important to understand what determines the thermoregulatory effects of TRPV1 antagonists in our own species. We already know that some TRPV1 antagonists, *e.g.*, CPZ and JYL1421, affect thermoregulation in a species-specific manner (see Section 2.1 above). We also know that at least some TRPV1 antagonists affect the TRPV1 channel in a species-specific manner as well and, hence, have different activation-mode pharmacological profiles against the TRPV1 channel in different species. A famous example is the TRPV1 antagonist AMG8562, which was the first one reported to cause analgesia without hyperthermia in rats (Lehto et al., 2008). It is thought to cause no hyperthermia in this species, because it does not inhibit proton activation of rTRPV1. However, if this assumption is true, the success of AMG8562 in causing hyperthermia-free analgesia in rats cannot be reproduced in humans – this antagonist is a potent inhibitor of all modes of activation of hTRPV1, including the proton mode (Lehto et al., 2008). Furthermore, the regulation of thermoregulatory responses in rats and humans is not likely to be identical; at the very least, rats do not sweat, whereas humans do not use tail-skin vasodilation or thermoregulatory salivation, and neither do humans rely on nonshivering thermogenesis in brown fat to the same extent as rats do (Romanovsky, 2018). Being several hundred times heavier than rats, humans take advantage of substantial thermal inertia and, accordingly, do not face the problem of readily losing the constancy of T_b (homeothermia) in view of environmental perturbations, at least not to the same extent as small rodents do. Consequently, peripheral thermal signals (including those mediated by TRPV1) are expected to play a smaller role in humans than in rodents (Romanovsky, 2014, 2018).

Last but not least, TRPV1 has different sensitivity to both temperature and chemical ligands in different species. For example, a TRPV1 isoform found in vampire bats is activated by temperatures as low as 30°C, which presumably makes TRPV1 a radiant-heat sensor for the detection of warm-blooded prey (Gracheva et al., 2011). On the other extreme, Bactrian camels express a TRPV1 ortholog that is not activated by temperature as high as 46°C (Laursen, Schneider, Merriman, Bagriantsev, & Gracheva, 2016), which, we think, is a genetic adaptation to high surface temperatures in camels' habitat during summer. Another dramatic example refers to TRPV1 sensitivity to CAP. Avian TRPV1 is not sensitive to CAP (Nagy & Rang, 2000), which allows birds to feed on spicy, pungent, CAP-rich fruits such as hot chili peppers. In contrast, most mammals, including mice (Garami et al., 2011), do not consume hot chili. In fact, CAP-containing repellants are used to protect crops from browsing by many species of mammalian pests (Romanovsky, 2015). In each of these examples, a pharmacological blockade of TRPV1 would have a distinct effect.

Based on the above, it is quite possible that TRPV1 antagonists cause their thermoregulatory effect somewhat differently in humans than in

Table 3
Characteristics of clinical studies of TRPV1 antagonists

Compound (company)	Patient or volunteer population	Thermometry site	Thermal effect	ClinicalTrials.gov identifier	Reference(s)
ABT-102 (Abbott)	Healthy male and female volunteers, 18-55 years	Oral	Increase in T_b	NCT00854659	Rowbotham et al., 2011
ABT-102 (Abbott)	Healthy volunteers	Oral or abdominal	Increase in T_b	Not reported	Othman et al., 2013
ABT-102 (Abbott)	Healthy male volunteers, 18-60 years	Aural	Dose-dependent increase in T_b	Not reported	Schaffler et al., 2013
AMG 517 (Amgen)	Patients with pain due to molar extraction	Oral and tympanic	Plasma concentration-dependent increase in T_b	Not reported	Gavva et al., 2008
AZD1386 (AstraZeneca)	Patients with pain due to lower "wisdom tooth" extraction	T_b not measured	T_b not measured	NCT00672646	ClinicalTrials.gov
AZD1386 (AstraZeneca)	Healthy volunteers	T_b not measured	T_b not measured	NCT00692146	ClinicalTrials.gov
AZD1386 (AstraZeneca)	Patients with post-traumatic or postherpetic neuralgia	T_b not measured	T_b not measured	NCT00976534	ClinicalTrials.gov
AZD1386 (AstraZeneca)	Patients with pain due to third molar extraction	Oral	Increase in T_b	Not reported	Quiding et al., 2013
AZD1386 (AstraZeneca)	Patients with knee osteoarthritis	T_b not measured	T_b not measured	NCT00878501	Miller, Bjornsson, Svensson, & Karlsten, 2014
AZD1386 (AstraZeneca)	Healthy male volunteers	Not reported	Dose-dependent increase in T_b	NCT00711048	Krarup et al., 2011
AZD1386 (AstraZeneca)	Patients with reflux disease responsive to proton-pump inhibitors	Not reported	Increase in maximum T_b	NCT01019928	Krarup et al., 2013
DWP05195 (Daewoong)	Patients with postherpetic neuralgia	T_b not measured	T_b not measured	NCT01557010	ClinicalTrials.gov
DWP05195 (Daewoong)	Healthy male volunteers	Not reported	Dose-dependent increase in T_b (tendency)	NCT00969787 (single dose) NCT01094834 (multiple doses)	Lee et al., 2017
GRC-6211 (Glenmark, Eli Lilly)	Patients with osteoarthritic pain, incontinence, or neuropathic pain	T_b not measured	T_b not measured	Not reported	Myers, 2008
JNJ-38893777 (Johnson & Johnson)	Healthy male volunteers	Oral	Small increase in T_b	Not reported	Manitpisitkul et al., 2015
JNJ-39439335 (Johnson & Johnson)	Healthy male volunteers	T_b not measured	T_b not measured	NCT01006304	ClinicalTrials.gov
JNJ-39439335 (Johnson & Johnson)	Healthy male volunteers	Oral	Small increase in T_b	Not reported	Manitpisitkul et al., 2016
JNJ-39439335 (Johnson & Johnson)	Healthy male volunteers	Oral	No clinically meaningful increase in T_b	Not reported	Manitpisitkul, Shalayda, Russel, Sanga, Solanki, et al., 2018
JNJ-39439335 (Johnson & Johnson)	Healthy male volunteers (Part 1); male and female patients with knee osteoarthritis (Part 2)	Oral	Plasma concentration-independent increase in T_b	Not reported	Manitpisitkul, Flores, et al., 2018
JNJ-39439335 (Johnson & Johnson)	Healthy male volunteers	Oral	Small increase in T_b	Not reported	Manitpisitkul, Shalayda, Russel, Sanga, Williams, et al., 2018
JNJ-39439335 (Johnson & Johnson)	Patients with knee osteoarthritis	Not reported	T_b increased in one patient (of 33 patients)	Not reported	Mayorga et al., 2017
MK-2295, or NGD-8243 (Merck)	Patients with postoperative dental pain	T_b not measured	T_b not measured	NCT00387140	ClinicalTrials.gov
MR-1817 (Mochida)	Healthy adult volunteers	T_b not measured	T_b not measured	NCT00960180	ClinicalTrials.gov
NEO6860 (NEOMED, Convance)	Healthy male volunteers	Gastrointestinal (ingestible transmitter)	No clinically significant change in T_b	NCT02337543	Brown et al., 2017
NEO6860 (NEOMED)	Adult patients with pain due to knee osteoarthritis	Oral	No increase of more than 1°C in T_b	NCT02712957	Arsenault et al., 2018
PAC-14028, or asivatrep (Amorepacific)	Patients with dermal pruritus	T_b not measured	T_b not measured	NCT02052531	ClinicalTrials.gov
PAC-14028, or asivatrep (Amorepacific)	Patients with dermal pruritus	T_b not measured	T_b not measured	NCT02565134	ClinicalTrials.gov
PAC-14028, or asivatrep (Amorepacific)	Patients with erythema-telangiectatic or papulopustular rosacea	T_b not measured	T_b not measured	NCT02052999	ClinicalTrials.gov
PAC-14028, or asivatrep (Amorepacific)	Patients with mild-to-moderate atopic dermatitis	T_b not measured	T_b not measured	NCT02757729 NCT02583022 NCT02965118	ClinicalTrials.gov
PAC-14028, or asivatrep (Amorepacific)	Patients with mild-to-moderate seborrheic dermatitis of the face	T_b not measured	T_b not measured	NCT02749383	ClinicalTrials.gov
PAC-14028, or asivatrep (Amorepacific, Seoul National University Hospital)	Healthy male volunteers	T_b not measured	T_b not measured	NCT02309008	ClinicalTrials.gov
PAC-14028, or asivatrep (Amorepacific)	Healthy male volunteers	T_b not measured	T_b not measured	NCT01264224	ClinicalTrials.gov

Table 3 (*continued*)

Compound (company)	Patient or volunteer population	Thermometry site	Thermal effect	ClinicalTrials.gov identifier	Reference(s)
PAC-14028, or asivatrep (Amorepacific)	Healthy adult volunteers	T_b not measured	T_b not measured	NCT01638117	ClinicalTrials.gov
PAC-14028, or asivatrep (Amorepacific)	Patients with rosacea	T_b not measured	T_b not measured	NCT02583009	ClinicalTrials.gov
PAC-14028, or asivatrep (Amorepacific)	Pediatric patients with atopic dermatitis	T_b not measured	T_b not measured	NCT02748993	ClinicalTrials.gov
PHE-377 (PharmEste)	Patients with neuropathic pain	T_b not measured	T_b not measured	Not reported	Evans, 2011
SB-705498 (GlaxoSmith-Kline)	Patients with migraine	T_b not measured	T_b not measured	NCT00269022	ClinicalTrials.gov
SB-705498 (GlaxoSmith-Kline)	Patients with third molar extraction	Tympanic	Maximal change in T_b from baseline was 0.8 ± 0.5°C at 1,000 mg (baseline not reported)	NCT00281684	ClinicalTrials.gov
SB-705498 (GlaxoSmith-Kline)	Healthy adult volunteers	Tympanic	Not reported	NCT01476098 (oral)	ClinicalTrials.gov
SB-705498 (GlaxoSmith-Kline)	Healthy adult volunteers	T_b not measured	T_b not measured	NCT01673529 (topical)	ClinicalTrials.gov
SB-705498 (GlaxoSmith-Kline)	Patients with rectal pain	T_b not measured	T_b not measured	NCT00461682	ClinicalTrials.gov
SB-705498 (GlaxoSmith-Kline)	Healthy adult volunteers	T_b not measured	T_b not measured	Not reported	Chizh et al., 2007
SB-705498 (GlaxoSmith-Kline)	Patients with refractory chronic cough	Tympanic	No change in T_b	Not reported	Khalid et al., 2014
SB-705498 (GlaxoSmith-Kline)	Patients with seasonal allergic rhinitis	Oral	No change in T_b	NCT01424397	Bareille et al., 2013
SYL-1001 (Sylentis)	Patients with ocular pain due to dry-eye syndrome	T_b not measured	T_b not measured	NCT01776658 NCT01438281 NCT02455999	Benitez-Del-Castillo et al., 2016
V116517 (Purdue)	Subjects with moderate-to-severe pain due to knee osteoarthritis	T_b not measured	T_b not measured	NCT01688934	ClinicalTrials.gov
V116517 (Purdue)	Patients with moderate-to-severe postherpetic neuralgia	T_b not measured	T_b not measured	NCT01688947	ClinicalTrials.gov
V116517 (Purdue)	Healthy male volunteers	Oral	No change in T_b	NCT02695745	Arendt-Nielsen et al., 2016
XEN-D0501 (Xention, Ario)	Patients with chronic idiopathic cough	T_b not measured	T_b not measured	NCT02233699	ClinicalTrials.gov
XEN-D0501 (Xention, Ario)	Patients with refractory chronic cough	Not reported	Not reported	Not reported	Belvisi et al., 2017
XEN-D0501 (Xention)	Healthy male (Part 1) or male and female (Part 2) volunteers	Aural	Dose-dependent increase in T_b	Not reported	Round et al., 2011
XEN-D0501 (Xention, Ario)	Patients with chronic obstructive pulmonary disease	T_b not measured	T_b not measured	NCT02233686	Smith et al., 2017

rats. Nothing is known about the contributions of different modes of hTRPV1 activation to the thermoregulatory effect of TRPV1 antagonists in humans. By conducting the mathematical analyses presented below, we intended to fill this void.

3.2. Mathematical modeling: which modes of hTRPV1 activation contribute to the hyperthermic response in humans?

For the present work, we identified 87 published reports on human clinical trials of TRPV1 antagonists, of which 18 trials involved recording some measure of deep (core) T_b (Fig. S1). Using the sets of inclusion and exclusion criteria (Tables S1 and S2), we were able to select five studies for a mathematical-modeling analysis, and also for meta-analysis (see Section 3.3. below). The full set of data used for modeling analysis is shown in Table S3. The model, which is based on the one we developed earlier (Garami et al., 2010), is described in detail in Supplementary Methods.

The modeling analysis has found that the hyperthermic effect of TRPV1 antagonists in humans is the most sensitive to the extent of hTRPV1 blockade in the proton activation mode. The sensitivity coefficient of the hyperthermic response to potency changes in the proton mode (mean ± SE) is 1.37 ± 0.00 ($P = 2.48 \times 10^{-39}$). In this respect, the hyperthermic response to TRPV1 antagonists in humans is similar to that in rats: in both species, potent blockers of proton activation cause an increase in T_b (Fig. 10). While preparing our recent study (Garami, Pakai, et al., 2018), we already had the information about the importance of the proton activation mode, based on preliminary results of our modeling analysis presented here, and we used these results to explain the phenomenon that was not well understood. It was reported that the TRPV1 antagonist V116517 potently blocked rTRPV1 activation by protons and caused hyperthermia in rats (Tafesse et al., 2014), but yet it did not cause hyperthermia in a human clinical trial, while potently blocking hTRPV1 activation by protons (Arendt-Nielsen et al., 2016). We proposed (Garami, Pakai, et al., 2018) that this discrepancy could be explained by the use of subthreshold (for causing hyperthermia) doses of V116517 in that particular trial, and that V116517 is an intrinsically hyperthermic (not neutral) compound. The data presented in the present work (Fig. 9B) show that our hypothesis is correct.

The present analysis has also found that, differently from the hyperthermic response in rats (which is insensitive to potency changes in the heat activation mode), the response in humans is highly sensitive to the antagonist's potency in the heat mode. For the human response, the sensitivity coefficient to potency changes in the heat mode is 1.09 ± 0.00 ($P = 1.92 \times 10^{-39}$). As for the CAP mode of activation, blocking it is not important for the hyperthermic response in either rats or humans. In humans, the response sensitivity to potency changes in the CAP mode is negative and six times lower in magnitude than the sensitivity in the proton activation mode. The sensitivity coefficient of the human response to potency changes in the CAP mode is -0.23 ± 0.00 ($P = 4.71 \times 10^{-38}$).

In addition to the set of mean sensitivity coefficients listed above and illustrated in Fig. 10, we have also found the best-fitted set of sensitivity

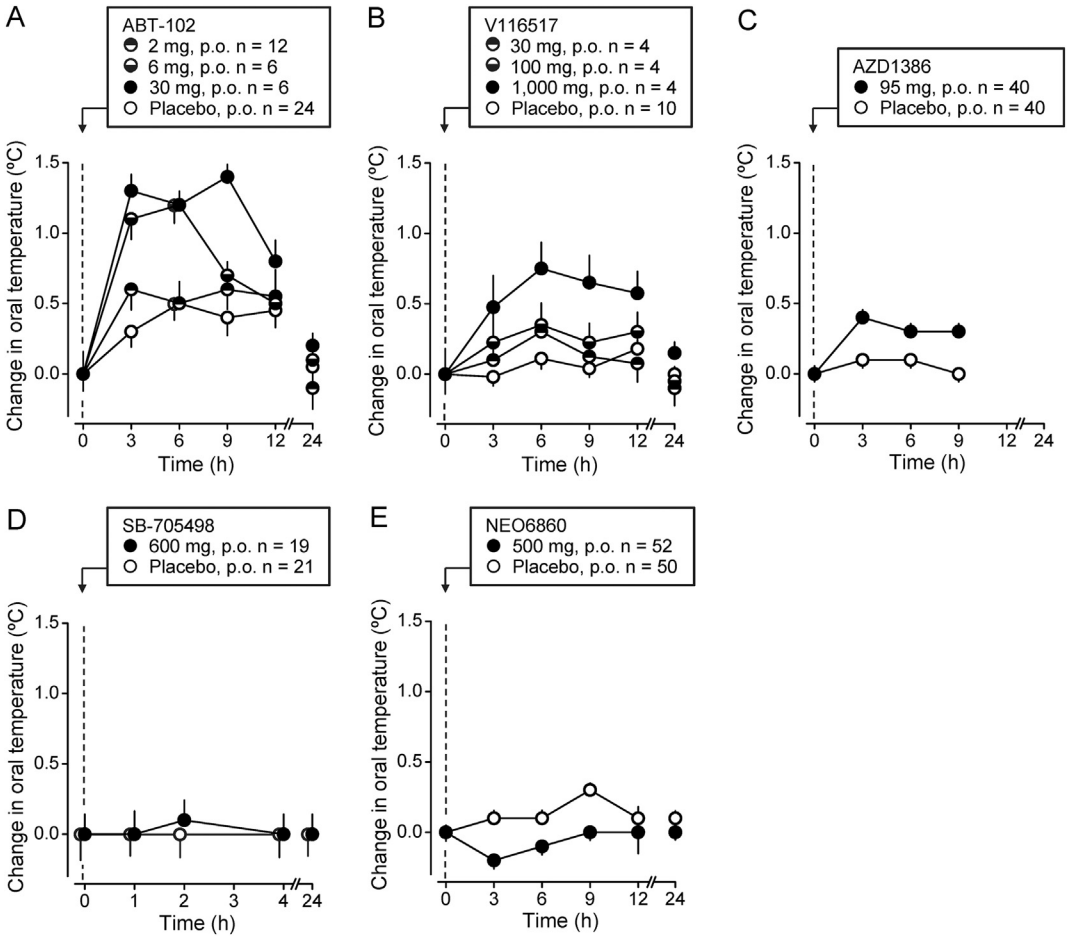


Fig. 9. Deep T_b responses of humans to TRPV1 antagonists vary from high hyperthermia to low hyperthermia, no effect, or hypothermia. A) Effect of ABT-102 (2, 6, and 30 mg/kg, p.o.) or placebo on oral temperature. B) Effect of V116517 (30, 100, and 1,000 mg/kg, p.o.) or placebo on oral temperature. C) Effect of AZD1386 (95 mg/kg, p.o.) or placebo on oral temperature. D) Effect of SB-705498 (600 mg/kg, p.o.) or placebo on oral temperature. E) Effect of NEO6860 (500 mg/kg, p.o.) or placebo on oral temperature. Plotted for this work based on data from Othman et al. (2013) (A), Arendt-Nielsen et al. (2016) (see also Table S6) (B), Quiding et al. (2013) (C), Khalid et al. (2014) (D), and Arsenault et al. (2018) (E).

coefficients (by using a Monte Carlo technique to run the model with randomly generated parameters; see Mathematical model description, Supplementary Information). The best-fitted set includes the following sensitivity coefficient values: -0.21 (CAP mode), 1.25 (proton mode), and 1.00 (heat mode). With this set, the model accounts for a maximal share of the hyperthermic response variation: 83% ($r^2 = 0.83$). The best-fitted set complements the mean values in characterizing how the potency in each activation mode affects the hyperthermic response to TRPV1 antagonists in humans.

In addition to the high value of r^2, the high quality of data fitting in our model is demonstrated by the low values of the so-called "mismatch errors", *i.e.*, the differences between the hyperthermic response values measured in clinical trials for the 16 antagonist doses used in this analysis (Table S3) and the corresponding response values predicted by our model. In our current analysis, the mismatch error varies from -0.3 to 0.4°C; the quadratic mean is 0.2°C.

The more complex nature of the hyperthermic response to TRPV1 antagonists in humans, as compared to that in rats (dependence on two activation modes *vs.* one), is also evident from how varying the antagonist potency in different activation modes contributes to the statistical variance of the human hyperthermic response (Fig. 10). In rats, > 81% of the statistical variance of the hyperthermic response is determined by the proton mode; the contributions of both the heat mode and the CAP mode are negligible (~1% each); and the contribution of factors unaccounted for by our model is 16%. In humans, these contributions are: 35% for the proton mode, 37% for the heat mode, 10% for the CAP mode, and 17% for unaccounted factors.

The main finding of our analysis is that the hyperthermic response to TRPV1 antagonists depends on the activation-mode TRPV1 pharmacology differently (and in a more complex fashion) in the human than in the rat. In rats, of the three modes studied (CAP, proton, and heat), only the proton mode matters: the hyperthermic response is highly sensitive to potency changes in this mode and is totally insensitive to potency changes in the CAP or heat mode. Accordingly, only potent blockers of rTRPV1 activation by protons cause hyperthermia in rats. In humans, the hyperthermic response is highly sensitive to potency changes in the proton mode of hTRPV1 activation as well. But it is also sensitive, almost to the same extent, to potency changes in the heat mode. Hence, perhaps the most interesting and somewhat unexpected conclusion from our analysis is that TRPV1 plays different roles in thermoregulation in humans, as compared to rats. In rats, it mediates the tonic suppression of thermogenesis and skin vasoconstriction by protons (or proton-like stimuli) in the abdomen, perhaps in trunk muscles; it does not serve as a thermosensor to the thermoregulation system in this species, or at least not in young male adults. In humans, TRPV1 also seems to mediate the effect of acidity on thermoregulation, but, in addition to this, it is likely to play a role of a true thermal sensor for the thermoregulation system – detecting T_bs to drive thermoeffector responses. Our analysis suggests that, in order to be thermally neutral in humans, a TRPV1 antagonist should have low potency in both temperature and proton activation modes of hTRPV1.

Our model also predicts that the strongest hyperthermic response in humans is induced by TRPV1 antagonists that are highly potent inhibitors of hTRPV1 activation by both protons and heat. This prediction is

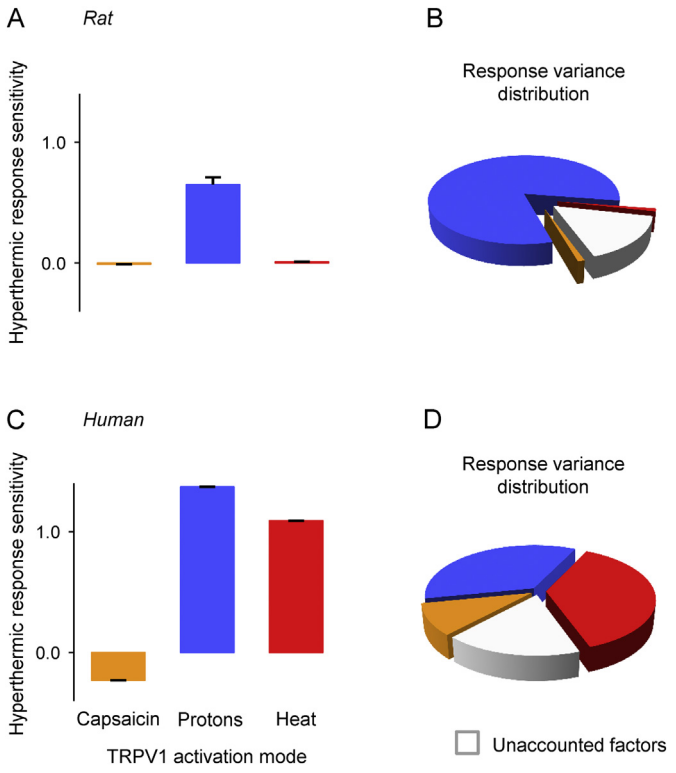


Fig. 10. Contribution of different modes of TRPV1 activation to the development of TRPV1 antagonist-induced hyperthermia in rats (A, B) and humans (C, D). TRPV1 antagonists differentially affect TRPV1 activation by CAP, protons, and heat; some TRPV1 antagonists cause the hyperthermic response. The sensitivity of the hyperthermic response to the antagonist potency in each of the three activation modes is presented as bars (mean ± standard error) for rats (A) and humans (C). While the hyperthermic response to TRPV1 antagonists in rats depends solely on the antagonist's potency to block TRPV1 activation by protons, the hyperthermic response in humans depends on the antagonist's potencies in both proton and heat modes. The pie charts depict the relative contributions of each mode (as well as of the factors unaccounted for by the model) to the statistical variance of the hyperthermic response in rats (B) and humans (D). Graphs are plotted from data reported by Garami et al. (2010) (A, B) or derived from the present mathematical analysis (C, D).

supported by the clinical trial of AMG 517 reported by Gavva et al. (2008). We could not use the Gavva et al. (2008) data in our analysis (Table S2), because the published report contained only the maximal values of T_b over a period of time (instead of the exact values at different time points). However, from the maximal T_b data presented, it is clear that AMG 517 causes marked hyperthermia in humans already at the very low dose of 2 mg (~5 μmol). AMG 517 is a highly potent blocker of hTRPV1 activation by both protons and heat; the corresponding IC_{50} values are 0.6 and 1.3 nM, respectively [Table S2; also see Gavva, Bannon, Surapaneni, et al. (2007)].

Furthermore, T_b data reported for most of the TRPV1 antagonists with a known pharmacological profile against hTRPV1 seem to be compatible with our model results (Arsenault et al., 2018; Gavva et al., 2008; Othman, Nothaft, Awni, & Dutta, 2013; Quiding et al., 2013; Rowbotham et al., 2011). SB-705498 may be an exception, as this compound blocks all three activation modes of hTRPV1 with high (3-6 nM) potency (Gunthorpe et al., 2007), and yet, in the trial reported by Khalid et al. (2014), a high dose of this compound (600 mg, p.o.) failed to increase deep T_b (Fig. 9D). However, the TRPV1 channel occupancy in that trial (based on the peak plasma SB-705498 concentration) was only 45%, thus suggesting that the dose used might have been insufficient for triggering on-target effects, including hyperthermia.

While the modeling analysis presented here enables us to make unique conclusions about the relationship between the activation-mode pharmacological profiles of TRPV1 antagonists and their thermal effects in humans, some caution should be exercised when these conclusions are interpreted or applied.

First, the model processed pharmacological profiles obtained against hTRPV1 *in vitro* and thermal responses recorded in humans *in vivo*, with measurements performed by different groups and by different methodologies. We do not know whether the channel behaves similarly *in vitro* and *in vivo*. We also ignore any pharmacokinetics-related differences between different compounds.

A less obvious, but perhaps very important, reason for caution is that the model assumes that there are only three modes of TRPV1 activation, and that they are independent of each other. In reality, however, the TRPV1 channel can be activated not only by CAP (and other vanilloids and some endocannabinoids), protons (acidic pH), and heat, but also by the myriad of other endogenous and exogenous ligands, including – just to give some examples – divalent (*e.g.*, Ni^{2+}) and trivalent (*e.g.*, Gd^{3+}) cations, polyamines, basic pH, eicosanoids, phospholipids, and terpenoids (for review, see Calixto, Kassuya, Andre, & Ferreira, 2005; Nilius & Szallasi, 2014). In fact, TRPV1 is known as the polymodal receptor *par excellence* (Holzer, 2009). Furthermore, there is certainly a crosstalk between different modes of TRPV1 activation (Blumberg et al., 2011), *e.g.*, mild acidosis sensitizes TRPV1 to both CAP and heat (reviewed by Holzer, 2009). The interdependence of different modes of TRPV1 activation clouds any interpretations of our modeling results.

Last but not least, the inordinate promiscuity of the TRPV1 channel makes it utterly sensitive to its environment. Accordingly, the channel is likely to play different physiological roles with changes in conditions, even in the same species. Consequently, effects of TRPV1 antagonists can also be expected to differ under different conditions. In the study by Gram et al. (2019) in Zucker obese rats, the TRPV1 antagonist BCTC improved insulin secretion at a young age of 6 months, but did not have this effect at a more mature age of 9 months. In our study (Wanner et al., 2012), the hyperthermic effect of AMG 517 in aged (44 weeks) mice was very similar to that in young (12 weeks) mice, but the effect of the antagonist on systemic inflammation changed with aging from anti-inflammatory to proinflammatory. Furthermore, the effect of AMG 517 on mortality of systemic inflammation in aged mice depended on whether the inflammation was septic (induced by colonic ligation and puncture) or aseptic (induced by a high dose of bacterial lipopolysaccharide).

A sex-dependent effect of the TRPV1 antagonist ABT-116 on locomotor activity was found in dogs; this compound produced a stronger effect in males than in females (Malek et al., 2012). Sex-dependent differences in the effects of CPZ on audiogenic seizures in rats (Cho, Vaca, Miranda, & N'Gouemo, 2018) and on the urethral response to noxious stimulation in mice (Yoshiyama, Araki, Kobayashi, Zakoji, & Takeda, 2010) have also been reported. These differences may be due to the demonstrated effects of ovariectomy, pregnancy, and sex hormones (*viz.*, progesterone, estradiol, and prolactin) on the expression and activity of TRPV1 in mice and rats (Diogenes et al., 2006; Ortiz-Renteria et al., 2018; Payrits et al., 2017; Wu et al., 2010). Estradiol has also been shown to upregulate TRPV1 at the mRNA level in human sensory neurons derived from embryonic stem cells (Greaves, Grieve, Horne, & Saunders, 2014).

Some thermoregulatory effects of TRPV1 antagonists may also be sex-dependent. Based on our experiments with AMG0347 in male rats (Steiner et al., 2007), we concluded that TRPV1 antagonists readily recruit autonomic thermoeffectors in the hyperthermic response, but do not use behavioral regulation (we are now revisiting the latter conclusion in a new study). Based on our experiments with multiple TRPV1 antagonists in male rats (Garami et al., 2010; Steiner et al., 2007), we concluded that blocking thermal signals does not affect thermoregulation. To our surprise, our recent experiments have shown that several TRPV1 antagonists affect behavioral thermoregulation in female rats, possibly by blocking thermal signals (Romanovsky, 2019). Clearly, more studies are needed, and any results obtained with TRPV1 antagonists should be generalized with great care. Without having a model of a

biological phenomenon in one's head, it is impossible to further study this phenomenon in a systematic way, but one should always be ready to change the model as new experimental data arrive.

3.3. Human clinical trials: meta-analysis

In addition to mathematical modeling, we also conducted a meta-analysis of the data reported in the identified articles, using standard meta-analysis tools (see Supplementary Methods), in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews (Table S4) and Meta-Analysis Protocols (Moher, Liberati, Tetzlaff, & Altman, 2009). This analysis was registered with PROSPERO (CRD 42018095220).

We included the studies that reported deep T_b values in both TRPV1 antagonist-treated and placebo groups at least at two time points: (i) at or shortly before the drug (or placebo) administration and (ii) at 3 h after the time of administration (Table S1). Studies for which all the necessary information could not be obtained were excluded from the analysis (Table S2). In certain cases, we had to make limited assumptions or simplifications, as explained in Table S5. The demographic profiles of the studies are outlined in Table S6. For each included study, we calculated the change in deep T_b as a difference between the T_b values at 3 h after the drug (placebo) administration *vs.* at the time of administration (0 h); this is the T_b change that was used in the modeling analysis as the value *H* of the hyperthermic response. We then calculated the difference between the T_b changes induced by a drug and those induced by placebo (difference in means); we considered the latter difference to represent the thermal effect of the drug in our meta-analysis. For all doses, the differences in means were standardized (based on variances) to obtain standardized differences in means (SDMs). The SDMs with 95% confidence intervals (CIs) were used as primary measures of effect size and are presented as a "forest plot" (Fig. 11). We considered each TRPV1 antagonist to be either mode-nonselective (ABT-102, AZD1386, and V116517) or mode-selective (NEO6860). To characterize the non-selective group, SDMs for the corresponding antagonists were weighted based on sample size and inversed variance.

All three antagonists in the mode-nonselective group caused hyperthermia, which was dose-dependent for those compounds that were administered at multiple doses, *viz.*, ABT-102 and V116517 (Fig. 11). The highest hyperthermic effect (SDM: 2.5; CI: 1.4-3.5) occurred in the group treated with the 86 μmol dose of ABT-102. The mean thermal effect of all doses of all mode-nonselective TRPV1 antagonists in the analyzed clinical trials was an SDM of 1.2 (CI: 0.9-1.6; $P < 0.001$). These results of meta-analysis agree with our mathematical-modeling results in that the blockade of the proton and heat activation modes of the hTRPV1 channel, as typically observed for polymodal TRPV1 antagonists, results in hyperthermia.

NEO6860, the only mode-selective TRPV1 antagonist that we were able to include in our analysis, did not cause hyperthermia at the dose used (1.2 mmol) but, instead, decreased the deep T_b (SDM: -0.7; CI: -0.3 to -1.1; $P < 0.001$). The lack of hyperthermia in response to NEO6860 agrees with our mathematical model, since this compound blocks neither proton nor heat activation of hTRPV1. The hypothermic effect of NEO6860 could be explained by the reported partial agonistic activity of this compound against hTRPV1 (Arsenault et al., 2018). TRPV1 agonists are well-known to cause hypothermia (Romanovsky et al., 2009; Szolcsanyi, 2015). TRPV1 antagonists with partial TRPV1 agonistic properties, such as 5'-iodo-RTX (Blumberg et al., 2011; Dogan et al., 2004; Shimizu, Iida, Horiuchi, & Caterina, 2005) or AbbVie's Compound 3 (Gomtsyan et al., 2015), also cause hypothermia. As a limitation of our meta-analysis, it should be mentioned that we analyzed only one dose of a single mode-selective drug; this analysis should be repeated as more data become available.

4. Tackling the thermal effects of TRPV1 antagonists: approaches to drug development

4.1. Designing thermally neutral antagonists based on mode-selective TRPV1 pharmacology

Perhaps the best solution would be to design a TRPV1 antagonist that possesses sufficient efficacy as an analgesic (or in respect to any other desired effects) but does not cause any of the on-target adverse effects on T_b, *i.e.*, hypo- or especially hyperthermia. Based on the current work, the thermal effects are likely to be minimal in humans for the compounds that do not block hTRPV1 activation by protons and temperature, even if they are still potent blockers of the channel activation by CAP. If the proton- and heat-blocking potencies are low enough,

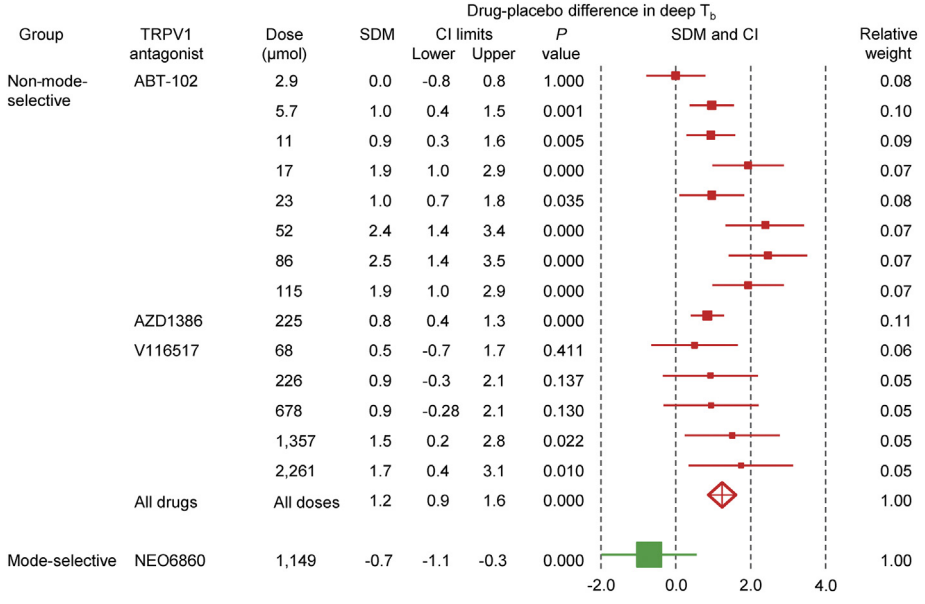


Fig. 11. Forest plot of the thermal effects of TRPV1 antagonists. For each antagonist dose, an SDM (a measure of the T_b response) and CI were calculated as described in the text (section 3.3) and are shown in the figure with a square and a horizontal bar, respectively. The area of the square is proportional to the sample size and inversed variance. The rhombus refers to the weighted mean SDM for the mode-nonselective group; the vertical diagonal of the rhombus points at the SDM value, whereas the horizontal diagonal represents the CI. Red symbols refer to mode-nonselective TRPV1 antagonists; the green square and bar refer to the selective antagonist NEO6860.

while the CAP-blocking potency is high enough, such a compound or biological has a chance of possessing sufficient efficacy (by potently blocking hTRPV1 activation by vanilloids) without affecting T_b. Rational design of therapeutics with this activation-mode profile clearly pushes the current boundaries of molecular pharmacology. Nonetheless, there are hints from TRPV1 mutational data that this may be possible. Early studies of TRPV1 orthologs showed that residues in the vanilloid-binding pocket could be mutated (Tyr511Ala and Ser512Tyr), which would selectively disrupt the CAP sensitivity, while retaining high sensitivity in the heat and proton activation modes (Jordt & Julius, 2002). Similarly, mutations in the pore turret of murine TRPV1 can selectively abrogate heat activation without impacting CAP activation (Cui et al., 2012), while distinct but proximal mutations have been shown to selectively disrupt proton activation (Jordt et al., 2000). More recently, random scanning mutagenesis of rTRPV1 has identified hundreds of specific mutations across the TRPV1 protein that retain either CAP- or heat-selective activation – although it is unclear how these mutations impact proton activation (Sosa-Pagan, Iversen, & Grandl, 2017). Taken together, these data prove that TRPV1 activation involves at least some stimuli-specific mechanismic differences. Leveraging these differences in gating-coupling modes of TRPV1 activation can potentially be instrumental for developing therapeutics. Indeed, multiple TRPV1 antagonists with various degrees of mode-selectivity (second-generation antagonists) have been synthesized, and some even tested in humans (*vide supra*).

There are two main obstacles for this approach. First, limited by the extent of the current knowledge, rational engineering of mode-selective TRPV1 compounds remains at an early stage. Second, there may be an intrinsic limit on how much the overall activation of TRPV1 can be blocked (for therapeutic purposes) without blocking channel activation by protons and temperature (to prevent thermoregulatory side effects). Reflecting this inherent limitation, the reasons for failure of TRPV1 antagonist clinical trials are now different than they were a decade ago. The early trials were conducted with first-generation (mode-nonselective) antagonists, and they typically failed due to the adverse hyperthermic effect; the trial with AMG 517 being a well-known example (Gavva et al., 2008). In contrast, several more recent trials were conducted with second-generation (mode-selective) TRPV1 antagonists, and some of these trials did not demonstrate the anticipated level of analgesia; trials of NEO6860 (Arsenault et al., 2018) being an example.

While rational engineering of TRPV1 antagonists with a desired profile is maturing, less efficient tools can also be used to achieve the same results, *e.g.*, "random" synthesis and *in-vitro* pharmacological screening. Based on publications, several thermally neutral, mode-selective TRPV1 antagonists have been (or were) considered by pharmaceutical companies for further development, including A-1165442 and A-1115760 by Abbott Laboratories and AbbVie (Reilly et al., 2012; Voight et al., 2014), AS1928370 (ASP8370) by Astellas Pharma (Oka et al., 2018; Watabiki, Kiso, Kuramochi, et al., 2011; Watabiki, Kiso, Tsukamoto, Aoki, & Matsuoka, 2011), and NEO6860 by NEOMED Institute (Arsenault et al., 2018; Brown et al., 2017).

4.2. Using therapeutic doses that are subthreshold for the hyperthermic response

In the section above, we discussed development of TRPV1 antagonists that are thermally neutral, meaning that they do not cause a thermal response in the entire dose range that can be reasonably expected to be used. However, the developers will always face conflicting demands: a thermally neutral antagonist, at least according to our model, should not block heat and proton activation of hTRPV1, whereas in order to be an efficacious analgesic (or whatever the desired therapeutic effect might be), it should potently block TRPV1 activation, sometimes in all modes. As a result, "compromise" antagonists are likely to emerge that exhibit some antagonism in non-CAP modes of activation at the price of causing hyperthermia at higher doses. Finding such a compromise, *i.e.*, using potentially hyperthermic antagonists at doses that are subthreshold for causing hyperthermia, can be viewed as a separate approach for dealing with the thermoregulatory side effects. This approach exploits differential sensitivity of the therapeutic *vs.* side effects to changes in exposure; it is also known as the therapeutic index-based approach. As evident from the literature, this approach might have been explored by Purdue Pharma in connection with the development of V116517, as discussed in section 3.2 (also see Fig. 9B). Since in an earlier trial in humans the TRPV1 antagonist XEN-D0501 caused dose-dependent hyperthermia (Round et al., 2011), one could speculate that the same approach may be used by the young pharmaceutical company Pila Pharma, which is developing XEN-D0501 for treating obesity and diabetes (Gram et al., 2017). Pila Pharma is also likely to be taking advantage of the approach described in the next section.

4.3. Taking advantage of the tachyphylaxis phenomenon

Another strategy to minimize the hyperthermia is by taking advantage of the fact that the hyperthermic effect of some TRPV1 antagonists fades away with repeated administration, whereas the desired effect (*e.g.*, analgesic) may not undergo such an attenuation (Gavva, Bannon, Hovland Jr., et al., 2007). The fading of the effect with repeated administration is called desensitization, or tachyphylaxis; we will use the latter term – to avoid confusion with CAP- or RTX-induced desensitization discussed in previous sections. In different animal models it has been shown that repeated dosing of AMG 517, AMG8163 (Gavva, Bannon, Hovland Jr., et al., 2007) or ABT-102 (Honore et al., 2009) can favorably shift the ratio of the desired effect (analgesia) to the adverse effect (hyperthermia). It is speculated that the selective modulation of some (but not other) effects of TRPV1 antagonists with repeated dosing can be due to the antagonist-sensitive regulation of the subcellular distribution of TRPV1, which can lead to relative strengthening or weakening of TRPV1-mediated responses generated from different locations within a cell (Johansen, Reilly, & Yost, 2006). While the exact molecular mechanisms of the effect-specific tachyphylaxis seen at the whole-body level are unknown, it is interesting that rTRPV1 desensitization *in vitro* (revealed as reduced ion conductance) has different mechanisms when caused by different stimuli. Whereas CAP-induced desensitization is Ca^{2+}- and calmodulin-dependent (Rosenbaum, Gordon-Shaag, Munari, & Gordon, 2004) and at least partially reversible (Numazaki et al., 2003), heat-induced desensitization is independent of either Ca^{2+} or calmodulin and, on the experimental time scale, is irreversible (Sanchez-Moreno et al., 2018).

Repeated administration of TRPV1 antagonists was also tested in human clinical trials. In the trial by Amgen (Gavva et al., 2008), the hyperthermic response to the highest dose (10 mg) of AMG 517 (but not to a lower dose of 2 or 5 mg) was attenuated with repeated antagonist administration (Fig. 12A). In the trials conducted by Abbott Laboratories, the hyperthermic response to any dose of ABT-102 used (1, 2, or 4 mg, twice a day) attenuated gradually with repeated dosing and faded by day 7 [Fig. 12B; also see Rowbotham et al. (2011)]. In the trial reported by Round et al. (2011), XEN-D0501 caused a dose-dependent core T_b increase, which was greatest on the first day of dosing but rapidly attenuated thereafter for all doses tested (1, 2.5, and 5 mg, twice a day) and, by day 14, was 0.3°C or less (as compared to the placebo group) for all doses studied.

A priori, any pharmaceutical company that deals with a chronic condition, thus requiring repeated drug administration, and works on TRPV1 antagonists with an inherent hyperthermic activity may try to use a tachyphylaxis protocol, whether by desire or as an added benefit of repeated dosing. Purdue Pharma conducted clinical trials of their TRPV1 antagonist V116517 (NCT01688934, 2012; NCT01688947, 2012). According to R. Kapil and D. J. Kyle (unpublished observations), the company saw a rapid abatement of hyperthermia with repeated dosing of V116517 in laboratory animals and humans alike, thus

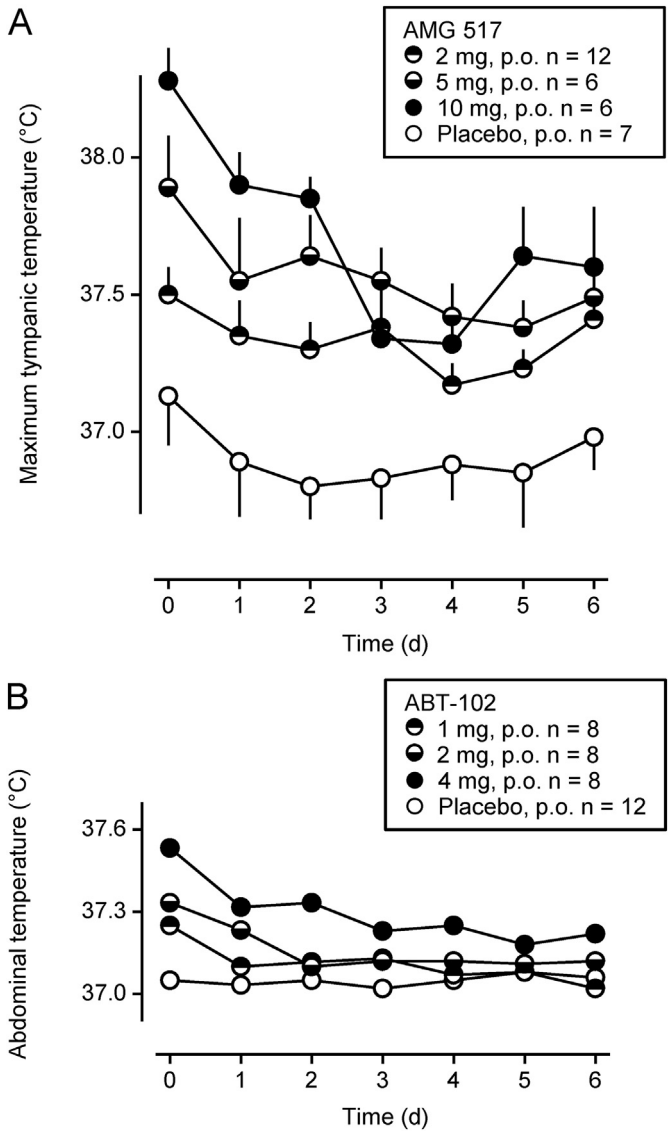


Fig. 12. The magnitude of the hyperthermic response to TRPV1 antagonists decreases with subsequent administration in humans. A) The effect of AMG 517 (2, 5, and 10 mg/kg, p.o., twice a day) or placebo on maximum tympanic temperature is shown for the first 7 days of treatment (days 0-6). B) The effect of ABT-102 (1, 2, and 4 mg/kg, p.o., twice a day) or placebo on abdominal temperature is shown for the first 7 days of treatment. Plotted for this work based on data from Gavva et al. (2008) (A) and Rowbotham et al. (2011) (B).

reducing the possible safety concerns for the drug. In addition to Purdue Pharma, several other companies, including Amgen, Daewoong Pharmaceutical, Pila Pharma, and Abbott Laboratories exercised (or at least considered) this approach, as is evident from the published reports (Gavva et al., 2008; Lee et al., 2017; Round et al., 2011; Rowbotham et al., 2011).

4.4. Combining a TRPV1 antagonist with an antihyperthermic drug

The low-hanging fruit was picked first. Several TRP programs at pharmaceutical companies considered combining a hyperthermic TRPV1 antagonist with some of the most obvious choices for drugs decreasing T_b – conventional antipyretics. Amgen published some research on this topic. Amgen's first report (Gavva, Bannon, Hovland Jr., et al., 2007) described an attempt of combining the TRPV1 antagonist AMG8163 with acetaminophen, an active constituent of Tylenol (and of more than 500 other over-the-counter and prescription medicines used to treat fever and pain). It appeared that acetaminophen did attenuate the hyperthermia induced by AMG8163 but only at a super high, hypothermia-inducing dose of 300 mg/kg, which is analogous to a 21-g bolus dose for a 70-kg human. Lower doses of acetaminophen that are normally used to treat fever (and that do not affect T_b in afebrile – without fever – conditions) do not diminish the hyperthermic response to AMG8163 in rats (Gavva, Bannon, Hovland Jr., et al., 2007). Nor do they affect the hyperthermic response to AMG 517 in human patients undergoing molar extraction (Gavva et al., 2008).

These negative results should not come as a surprise, because acetaminophen is thought to block fever by inhibiting cyclooxygenase (Engstrom Ruud et al., 2013), the key enzyme in the synthesizing cascade of prostaglandin E_2 (Ivanov & Romanovsky, 2004). While prostaglandin E_2 is the principal mediator of fever (Garami, Steiner, & Romanovsky, 2018), there are no data on prostaglandin involvement in the genesis of the hyperthermic response to TRPV1 antagonists. The noninvolvement of prostaglandin E_2 would explain the resistance of AMG8163- or AMG 517-induced hyperthermia to acetaminophen. On the other hand, the attenuation of AMG8163-induced hyperthermia in rats by the super high dose of acetaminophen (Gavva, Bannon, Hovland Jr., et al., 2007) is likely to be independent of TRPV1 and has been proposed to involve TRPA1 (Mirrasekhian et al., 2018), even though TRPA1 does not seem to play a role in T_b regulation in rats (de Oliveira et al., 2014). Alternatively, the hypothermic action of very high doses of acetaminophen can be, at least partially, due to the accumulation of acetaminophen metabolites, some of which exert a TRPV1-agonistic action (Eberhardt et al., 2017; Ohashi et al., 2017; Stueber et al., 2018).

A more fruitful approach can be combining a hyperthermic TRPV1 antagonist with a drug that blocks the sympathetic activation. Indeed, TRPV1 antagonists increase T_b by mounting the autonomic cold-defense responses: thermogenesis and skin vasoconstriction (Gavva et al., 2008; Steiner et al., 2007). These responses are sympathetically-driven not only when they are triggered by cold exposure or pyrogens (Morrison, 2011), but also when they are induced by TRPV1 antagonists, *e.g.*, AMG9810 (Alawi et al., 2015). The sympathetic mediation of TRPV1 antagonist-induced hyperthermia paves a way for combining a TRPV1 antagonist with a sympatholytic (sympathoplegic) drug, *i.e.*, a drug that opposes the downstream propagation of the neural signal in the sympathetic nervous system. The broad class of sympatholytics exploits a plethora of mechanisms and includes postsynaptic α- and β-adrenoreceptor antagonists, presynaptic α_2-adrenoreceptor agonists, catecholamine synthesis inhibitors, vesicular monoamine transporter inhibitors, and drugs with some other mechanisms of action (Stowe & Ebert, 2013). Based on the public information (Gomtsian, 2019), the pharmaceutical startup Synventa may be using the strategy described in this section. Synventa proposes that the α_2-adrenoceptor agonist lofexidine can prevent the hyperthermic effect of a TRPV1 antagonist and that using the two together will result in a thermally neutral analgesic combination.

4.5. Turning the adverse hyperthermia into a desired effect

Yet another approach to dealing with the adverse effect of TRPV1 antagonists on T_b would be to exploit their hyperthermic potential for a therapeutic purpose, *e.g.*, for counteracting hypothermia associated with general anesthesia in surgery (Schmidt, 2017) and possibly other types of hypothermia, especially when accompanied by pain or hyperalgesia. Anesthesia-associated hypothermia can be dramatic in magnitude (several degrees Celsius) and carries a significant risk of serious adverse effects, including coagulopathy and blood loss, as well as an increased propensity for wound infection (Ruetzler & Kurz, 2018). In animal models (Fig. 13), TRPV1 antagonists readily prevent or reverse anesthesia-associated hypothermia, while reducing the need for opioids for coping with postsurgical pain (Garami et al., 2017). As evident from the literature (Schmidt, 2017) and a published patent application

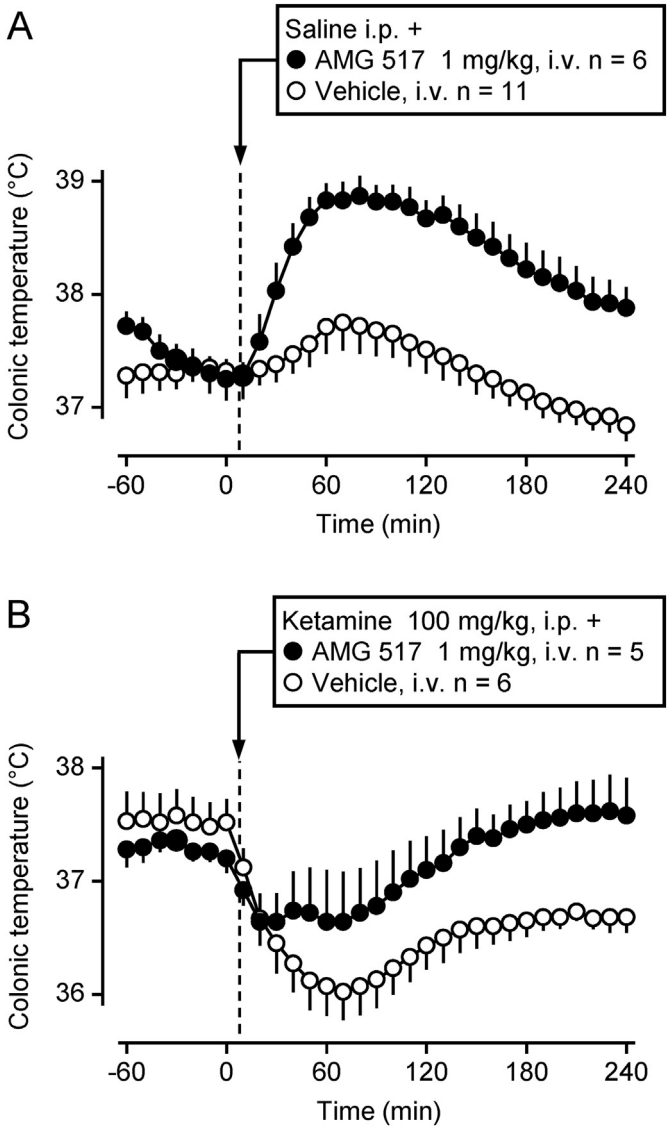


Fig. 13. AMG 517 causes hyperthermia in conscious rats but prevents hypothermia during anesthesia – without causing hyperthermia. A) The effect of AMG 517 (1 mg/kg, i.v.) or its vehicle on colonic temperature of rats pretreated with saline (i.p.) at a subneutral T_a of 23°C. B) The effect of AMG 517 (1 mg/kg, i.v.) or its vehicle on colonic temperature of rats pretreated with ketamine (100 mg/kg, i.p.) at a subneutral T_a of 23°C. Modified with permission from Garami et al. (2017).

(Patwardhan, Porreca, & Romanovsky, 2017), the strategy described in this section is used by the pharmaceutical startup Catalina Pharma.

5. Summary and conclusions

1. In rats and other laboratory animals, TRPV1 antagonists alter the level of T_b: most cause hyperthermia, whereas some produce hypothermia, and yet others exert no effect on thermoregulation. In general, the thermoregulatory effects of TRPV1 antagonists are dose-dependent. For some compounds, the effects are species-specific. The most common effect – hyperthermia – often fades with repeated dosing. Despite their diversity, all these thermoregulatory responses are likely to reflect an on-target (TRPV1-mediated) action of TRPV1 antagonists.
2. The TRPV1 protein is a species-specific channel that can be activated by a variety of stimuli, including (but not limited to) CAP and other vanilloids, protons (low pH), and heat. Activation of TRPV1 by different stimuli involves at least some mechanismic differences related to distinct portions on the channel structure and different loci of the molecule. These activation-mode-specific differences pave the way for developing mode-selective (mode-specific) TRPV1 antagonists. TRPV1 antagonists that potently block all activation modes are called mode-nonselective (or mode-nonspecific); they represent the first generation of TRPV1 antagonists. Second-generation (mode-selective) TRPV1 antagonists potently block the channel activation by CAP, but exert different effects (*e.g.*, potentiation, no effect, or low-potency inhibition) in either the proton mode or the heat mode, or both.
3. In young male rats, TRPV1 channels are not used as thermosensors for the thermoregulation system: thermal (T_b) signals that are mediated by TRPV1 do not normally drive thermoeffector responses in this species. Hence, blocking thermal activation by TRPV1 antagonists has no thermoregulatory effect in rats. Contrary to the widespread assumption, the mechanism of thermoregulatory effects of TRPV1 antagonists in this species does not involve blocking TRPV1-mediated thermosensation. Instead, TRPV1 antagonists affect thermoregulation by blocking the tonic TRPV1 activation by protons (or other ligands that share the proton activation mechanism) on sensory afferents somewhere in the trunk (abdomen), perhaps in the muscles. The hyper- and hypothermic responses share the same mechanism, which utilizes the acido-antithermogenic and acido-antivasoconstrictor reflexes. When the tonic activation of truncal TRPV1 channels is blocked (by hyperthermic antagonists) or potentiated (by hypothermic antagonists), the autonomic cold defenses (thermogenesis and cutaneous vasoconstriction) become either disinhibited or further inhibited, respectively, and either hyper- or hypothermia occurs. This mechanism may play a vital role during strenuous exercise by attenuating the inhibitory effect of T_b on physical performance.
4. If one were to design an "ideal" TRPV1 antagonist to treat pain in rats, such a compound would belong to the second generation and be a potent blocker of rTRPV1 activation by CAP and heat (to be an efficacious analgesic) but would not affect rTRPV1 activation by protons (to be free of the adverse effects on T_b).
5. Similar to their effects in laboratory rodents, TRPV1 antagonists alter the level of T_b in humans: most cause dose-dependent hyperthermia, whereas some may produce hypothermia. The hyperthermic effect often fades with repeated dosing.
6. The mathematical modeling used in the present work shows that the hyperthermic effect of a TRPV1 antagonist in humans depends on the compound's potency to block channel activation not only by protons, but also by heat. The connection between the hyperthermic response to TRPV1 antagonists and the heat mode of TRPV1 activation is present only in humans, not in rats. Similar to the hyperthermia in rats, the thermoregulatory response to a TRPV1 antagonist in humans does not depend on the compound's potency to block TRPV1 activation by CAP.
7. Based on the model analysis, we speculate that the role of TRPV1 in thermoregulation differs drastically between rats and humans. Whereas in rats TRPV1 channels modulate T_b *via*, most likely, pH signals from the trunk but are not used as thermosensors by the thermoregulation system, TRPV1 channels in humans may play both roles. The location of TRPV1 channels that sense T_bs (whether shell or core) to drive thermoeffector responses in humans is unknown and can be different from the location of the channels that are tonically activated by protons. Knowing that the skin plays a prominent thermosensory role in all species, at least some TRPV1 channels that mediate thermal signals to drive thermoeffectors in humans can be speculated to be located in the skin.

8. An “ideal” TRPV1 antagonist to treat pain in humans would belong to the second generation and be a potent blocker of hTRPV1 activation by CAP (to be an efficacious analgesic) but would not affect hTRPV1 activation by protons or heat (to be free of the adverse effects on T_b). The main concern about such an antagonist would be potentially a reduction in the level of efficacy. Such a drug would be most suited for treating pain caused exclusively through the CAP mode of TRPV1 activation – without any involvement of the heat and proton activation modes of activation; it is unclear whether such pain exists under natural conditions.
9. Our meta-analysis of the human-trial data has confirmed that the first-generation TRPV1 antagonists cause hyperthermia in humans, whereas the second-generation compounds may lack this effect.
10. The strategies of harnessing the thermoregulatory effects of TRPV1 antagonists in humans include: (i) creating inherently thermally neutral compounds based on mode-selective TRPV1 pharmacology, either by rational design or by more traditional approaches; (ii) creating potentially hyperthermic TRPV1 antagonists but with such profiles that would allow balancing the therapeutic effect with the adverse hyperthermic effect by using therapeutic doses that are subthreshold for the hyperthermia; (iii) taking advantage of the tachyphylaxis phenomenon (fading of the hyperthermic effect with repeated dosing) – in those cases when the desired therapeutic effect does not fade; (iv) combining a hyperthermic TRPV1 antagonist with an antihyperthermic drug (*e.g.*, a sympatholytic); and (v) turning the adverse hyperthermia into a desired effect, *e.g.*, by using TRPV1 antagonists to prevent anesthesia-associated hypothermia.

Declaration of Competing Interest

DAC is employed by NEOMED Institute. RK and DJK are employed by Purdue Pharma LP. AAR is an officer and director of Catalina Pharma Inc. and Zharko Pharma Inc.; he has consulted for TRPV1 programs at several pharmaceutical companies, and his laboratory conducted paid research on TRPV1 for Amgen Inc., Abbott Laboratories, and AbbVie Inc.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pharmthera.2020.107474.

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