

Perspective

Answering the Burning Question of How Transient Receptor Potential Vanilloid-1 Channel Antagonists Cause Unwanted Hyperthermia

SAMIR S. AYOUB, JOHN C. HUNTER, AND DANIEL L. SIMMONS

Centre for Biochemical Pharmacology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom (S.S.A.); and Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah (J.C.H., D.L.S.)

Abstract—The treatment of chronic pain with new therapies that lack the side effects of existing analgesics is one of medicine's great unmet needs. Toward this goal, antagonists of the transient receptor potential vanilloid-1 (TRPV1) channel have shown some promise. However, the development of these compounds has been hindered by an unpleasant on-target hyperthermic side effect. With compelling evidence,

the accompanying critical review by Romanovsky et al. (p. 228) regarding TRPV1 takes a position on the sites of action of TRPV1 agonists and antagonists on the thermoregulatory system that controls this side effect. From this comes insight on potential ways to overcome the unwanted hyperthermic action of TRPV1 antagonists.

The transient receptor potential vanilloid-1 (TRPV1)¹ channel is a nonselective cation channel expressed on the membrane of neurons that is activated by several stimuli, such as noxious heat, low pH, and vanilloid compounds such as capsaicin. In recent years, pharmaceutical companies have aggressively pursued TRPV1 antagonists to treat inflammatory and neuropathic pain, because TRPV1 is found on a subset of nociceptors sensitive to various pain stimuli, the response to which is attenuated in TRPV1 knockout mice (Caterina et al., 2000; Davis et al., 2000; Premkumar and Sikand, 2008). From a pharmaceutical standpoint, the stakes couldn't be higher, since the worldwide analgesic market in 2010

is estimated to be \$75 billion (Gharat and Szallasi, 2007).

However, as early as 1878, it was noticed that the substance responsible for the "hot" taste of red peppers, later isolated and identified as capsaicin, when introduced into the stomach, caused body temperature in dogs to fall, suggesting a role of the capsaicin-activated pathway in thermoregulation (Jancsó-Gábor et al., 1970). This hypothermic effect has now been validated over decades in numerous studies and organisms with various TRPV1 agonists termed vanilloids. Most importantly in terms of drug development, antagonism of TRPV1 by compounds of differing chemotypes has now been shown to cause serious hyperthermia in laboratory animals and in humans. This on-target side effect is not trivial, patients reaching temperatures as high as ~40°C (104°F), and has created an obstacle for the development of TRPV1 antagonists as therapeutic agents (Gavva et al., 2008).

The review article by Romanovsky et al. (2009) published in this issue is the much-anticipated thorough treatment that seeks to clarify the ever-confusing question of how TRPV1 functions in thermoregulation. Romanovsky et al. (2009) provide a holistic view of TRPV1 in which it does not act as a central, hypothalamic thermosensor for regulation of deep body temperature.

Rather, they present compelling evidence that TRPV1 channels function in a neural pathway that normally

Address correspondence to: Dr. Daniel L. Simmons, Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602. E-mail: dan_simmons@byu.edu

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¹ Abbreviations: TRPV1, transient receptor potential vanilloid-1; POA, preoptic area; AM404, *N*-(4-hydroxyphenyl)-arachidonylamide; AMG0347, (2*E*)-*N*-(7-hydroxy-5,6,7,8-tetrahydro-1-naphthalenyl)-3-(2-(1-piperidinyl)-6-(trifluoromethyl)-3-pyridinyl)-2-propenamide; AMG 517, *N*-(4-((6-(4-(trifluoromethyl)phenyl)-4-pyrimidinyl)oxy)-1,3-benzothiazol-2-yl)-acetamide; AMG8562, (2*E*)-*N*-((2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-4-yl)-3-(2-(1-piperidinyl)-4-(trifluoromethyl)phenyl)-2-propenamide; AMG7905, *N*-(6-(2-(cyclohexylmethyl)amino)-4-(trifluoromethyl)phenyl)-4-pyrimidinyl)-1,3-benzothiazol-6-amine.

responds to tonic nonthermal signals from the viscera (Fig. 1, right). These tonic signals result in the activation of TRPV1 channels to inhibit the cold defense mechanisms and suppress core body temperature. Tonic activation of visceral TRPV1 is proposed to be modulated in vivo by endovanilloids and certain stimuli, such as low pH, to evoke changes in core body temperature. This “illogical” pathway is not involved in responding to physiological temperatures; rather, it influences core body temperature in response to “illogical” or nonthermal stimuli. Examples of these types of stimuli are the reflexive changes in brown adipose tissue metabolism that occur upon stomach distension. The details of this physiological modulation by endogenous endovanilloids or other stimulators are largely unknown.

Exogenous TRPV1 agonists and antagonists, according to Romanovsky et al. (2009), inversely influence core body temperature according to where each type of compound acts on TRPV1 receptors in the tonic neuronal pathway. Antagonists work on peripheral neurons, whereas agonists work centrally at the preoptic area (POA) of the hypothalamus. Antagonists remove the tonic “brake” on body temperature exerted by this neural pathway, resulting in hyperthermia, whereas agonists, acting centrally, decrease temperature by stimulating TRPV1 on neurons that act to suppress the cold defense mechanisms (e.g., nonshivering thermogenesis and vasoconstriction) of the body (Fig. 1). By this scheme, TRPV1 agonists and antagonists are acting on only one of a number of integrated neural pathways that the body uses to sense changes, thermal and nonthermal, in its environment rather than on a hypothetical central hypothalamic neuronal site that governs the set point for core body temperature.

From a pharmacological standpoint, this is important in determining whether the severe hyperthermic side effect of TRPV1 antagonists can be avoided as well as determining how other pharmacologically important drugs work that change core body temperature, such as

acetaminophen. It has thus been proposed that the actions of acetaminophen on the thermoregulatory system (antipyresis and hypothermia), which are still poorly understood, may be mediated through activation of TRPV1. Högestätt et al. (2005) demonstrated that acetaminophen is converted to AM404, which is a hypothermic agent with potency for the activation of TRPV1 similar to that of capsaicin (De Petrocellis et al., 2000). Thus AM404 may be responsible for the hypothermic action of acetaminophen. However, less than 0.002% of acetaminophen is converted to AM404. This issue is anything but settled, and acetaminophen and other compounds may reveal additional neural target sites involved in thermoregulation.

Some readers will find the stance of Romanovsky et al. (2009) regarding the sites of action of TRPV1 agonists and antagonists to be controversial, because the authors, like other investigators, are faced with the fact that TRPV1 channels are widely distributed in the body on both neural and non-neural cells, such as mast cells, glial cells, and keratinocytes (Premkumar and Sikand, 2008). For example, TRPV1 is expressed in the terminal ends of afferent thinly myelinated A and unmyelinated C-fibers innervating many organs that include the skin, skeletal muscles, and visceral organs. In the brain, TRPV1 is also widely distributed, including, but not limited to, the thermoregulatory POA of the hypothalamus. The question, then, is which TRPV1 channels among the myriad of possibilities act to affect body temperature?

Romanovsky et al. (2009) pinpoint the site of agonist action to median preoptic nucleus neurons in the hypothalamus. They come to this conclusion based on pharmacological, neuron ablation, and desensitization studies, as well as in vitro TRPV1 analysis. Because median preoptic nucleus neurons are insensitive to temperature, they could not be hypothalamic thermosensors. Data implicating these neurons as the agonist site of action include the fact that capsaicin, the first known TRPV1 agonist, induces a hypothermic response when administered systemically. Direct administration of capsaicin into the POA at doses 25 times lower than those given systemically was found to be sufficient to induce hypothermia, strongly supporting a central site of action. More support for this argument comes from the observation that animals treated repeatedly with high doses of capsaicin or resiniferatoxin (RTX), a TRPV1 agonist, respond with desensitization of the TRPV1 channel and impairment of the hypothermic response. Specifically, rats pretreated intrahypothalamically with capsaicin demonstrated reduced sensitivity to systemically administered capsaicin to induce hypothermia due to POA neuronal death.

In contrast to agonists, the authors demonstrate that the site of TRPV1 antagonist-induced hyperthermia is not the brain, but the abdominal cavity (“viscera” in Fig. 1). To support this, they note that AMG0347 (a synthetic

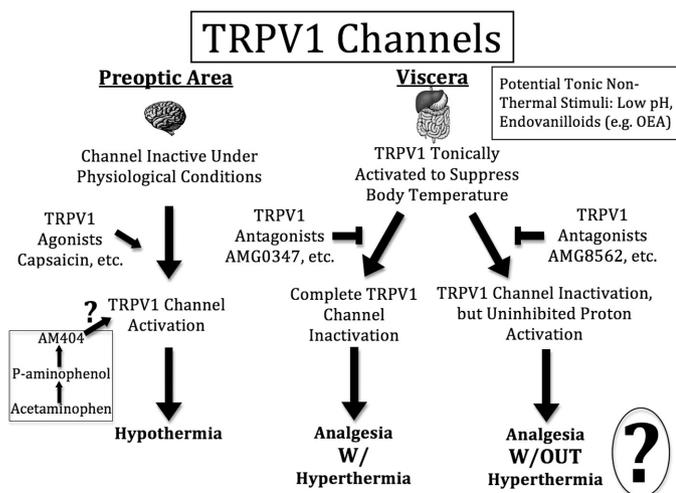


FIG. 1. Proposed sites of action of TRPV1 agonists and antagonists.

TRPV1 antagonist) administered intravenously at doses of 6 and 10 $\mu\text{g}/\text{kg}$ induced hyperthermia; however when administered intracerebroventricularly or intrathecally at a similar dose range it failed to induce hyperthermia. In addition, animals treated with 0.02 mg/kg RTX intraperitoneally, resulting in localized intra-abdominal desensitized TRPV1 channels, did not develop hyperthermia when treated with AMG0347. The localized intra-abdominal desensitization of TRPV1 is demonstrated by changes in satiety response to cholecystokinin and writhing response to intraperitoneal RTX with no changes to physiological responses associated with TRPV1 channels in other tissues such as the eyes, skin, thoracic cavity and brain (Steiner et al., 2007). These findings provide strong evidence for a visceral site of hyperthermic action of TRPV1 antagonists.

This is important from a therapeutic point of view because it allows the authors to propose a number of strategies to dissociate the therapeutic (analgesia) action of TRPV1 antagonists from their on-target side effect (hyperthermia). Two of these strategies have shown some promise. These include the fact that, upon repeated administration of antagonists, hyperthermia attenuates whereas analgesia ensues. This strategy, of course, exposes the patient to one or more unpleasant rounds of hyperthermia-inducing TRPV1 antagonist treatment before things presumably get better. They also note that in clinical trials with AMG 517, only high doses (10 mg) brought about subsequent attenuation of hyperthermia (Gavva et al., 2008).

The most promising strategy, however, is based on the fact that opening of the channel uses different gating mechanisms based on the agonist in question. Thus, different TRPV1 antagonists may block activation of the channel depending on the agonist. A good example that the authors use to illustrate this point is that capsaicin failed to antagonize TRPV1 when activated by protons and has not been reported to induce hyperthermia. It was later confirmed that an antagonist that

blocks TRPV1 activation by capsaicin and potentiates the activation induced by protons (instead of blocking) was likewise found to lack a hyperthermic action in rats (where it actually induced hypothermia instead). Such compounds include AMG8562 and AMG7905. Unfortunately, AMG8562 at least, is not expected to work in humans, because it does not inhibit proton activation of TRPV1 in rats, but it inhibits all modes of TRPV1 activation in human (Lehto et al., 2008). Until these compounds are tested in clinical trials, whether hyperthermia-free TRPV1 antagonists can be developed will remain hotly debated.

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