Transient Receptor Potential Vanilloid 1 Antagonists Prevent Anesthesia-induced Hypothermia and Decrease Postincisional Opioid Dose Requirements in Rodents

Andras Garami, M.D., Ph.D., Mohab Ibrahim, M.D., Ph.D., Kerry Gilbraith, B.S., Rajesh Khanna, Ph.D., Eszter Pakai, M.S., Alexandra Miko, M.D., Erika Pinter, M.D., Ph.D., D.Sc., Andrej A. Romanovsky, M.D., Ph.D., Frank Porreca, Ph.D., Amol M. Patwardhan, M.D., Ph.D.

ABSTRACT

Background: Intraoperative hypothermia and postoperative pain control are two important clinical challenges in anesthesiology. Transient receptor potential vanilloid 1 has been implicated both in thermoregulation and pain. Transient receptor potential vanilloid 1 antagonists were not advanced as analgesics in humans in part due to a side effect of hyperthermia. This study tested the hypothesis that a single, preincision injection of a transient receptor potential vanilloid 1 antagonist could prevent anesthesia-induced hypothermia and decrease the opioid requirement for postsurgical hypersensitivity.

Methods: General anesthesia was induced in rats and mice with either isoflurane or ketamine, and animals were treated with transient receptor potential vanilloid 1 antagonists (AMG 517 or ABT-102). The core body temperature and oxygen consumption were monitored during anesthesia and the postanesthesia period. The effect of preincision AMG 517 on morphine-induced reversal of postincision hyperalgesia was evaluated in rats.

Results: AMG 517 and ABT-102 dose-dependently prevented general anesthesia-induced hypothermia (mean ± SD; from 1.5° ± 0.1°C to 0.1° ± 0.1°C decrease; $P < 0.001$) without causing hyperthermia in the postanesthesia phase. Isoflurane-induced hypothermia was prevented by AMG 517 in wild-type but not in transient receptor potential vanilloid 1 knockout mice (n = 7 to 11 per group). The prevention of anesthesia-induced hypothermia by AMG 517 involved activation of brown fat thermogenesis with a possible contribution from changes in vasomotor tone. A single preincision dose of AMG 517 decreased the morphine dose requirement for the reduction of postincision thermal (12.6 ± 3.0 *vs.* 15.6 ± 1.0 s) and mechanical (6.8 ± 3.0 *vs.* 9.5 ± 3.0 g) withdrawal latencies.

Conclusions: These studies demonstrate that transient receptor potential vanilloid 1 antagonists prevent anesthesia-induced hypothermia and decrease opioid dose requirements for the reduction of postincisional hypersensitivity in rodents.
(ANESTHESIOLOGY 2017; XXX:00-00)

AVOIDANCE of intraoperative hypothermia and techniques to minimize postoperative pain compose two of the important tasks an anesthesiologist is responsible for. Intraoperative and perioperative hypothermia result from various factors, including the effects of general anesthesia itself (vasodilatation and inhibition of shivering-induced thermogenesis) and surgical conditions (wide operative fields, cold irrigation solutions, *etc.*).[1] Intraoperative and perioperative hypothermia can be associated with increased surgical site infections, increased bleeding, poor wound healing, and increased cardiovascular stress. Thus, intraoperative and perioperative hypothermia may contribute to increased morbidity and mortality associated with surgeries.[1] At the present time, the maintenance of perioperative normothermia relies on physical means of warming a patient (*e.g.*, forced-air warming blanket, heated intravenous solutions). However, these methods are inadequate in many

What We Already Know about This Topic

- Antagonists selective for the transient receptor potential vanilloid 1 receptor–ion channel can raise body temperature
- Transient receptor potential vanilloid 1 antagonists also have analgesic properties

What This Article Tells Us That Is New

- The selective transient receptor potential vanilloid 1 antagonists AMG 517 and ABT-102 dose-dependently prevented hypothermia during general anesthesia in rats
- These same drugs reduced incisional nociceptive sensitization

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). A.G. and M.I. contributed equally to this article.

Submitted for publication December 7, 2016. Accepted for publication July 11, 2017. From the Institute for Translational Medicine (A.G., E. Pakai, A.M.) and Department of Pharmacology and Pharmacotherapy (E. Pinter), Medical School, University of Pecs, Pecs, Hungary; Departments of Anesthesiology (M.I., K.G., F.P., A.M.P.), Pharmacology (M.I., K.G., R.K., F.P., A.M.P.), and Neuroscience (R.K.), University of Arizona, Tucson, Arizona; Systemic Inflammation Laboratory (FeverLab), Trauma Research, St. Joseph's Hospital and Medical Center, Phoenix, Arizona (A.A.R.).

 Anesthesiology 2017; XXX:00-00

<zdoi;10.1097/ALN.0000000000001812>

surgeries.[2–5] Moreover, according to some,[6,7] but not all,[8] the turbulent air flow from forced air may be associated with postsurgical infections.

Poor postsurgical pain control results in increased suffering, diminished function, hospital-related complications including infections, cardiovascular issues, and bleeding, all leading to longer in-hospital stays.[9,10] Moreover, a strong link exists between acute postsurgical pain intensity and the risk of development of chronic pain.[11] Management of postoperative pain relies primarily on opioids and nonsteroidal antiinflammatory drugs. Excessive opioid use in the perioperative phase is associated with increased neurologic and respiratory morbidities.[12] Nonsteroidal antiinflammatory drugs cause increased bleeding and negatively affect bone healing and kidney function.[13] Multiple anesthetic techniques and drugs have been evaluated as candidates for preemptive analgesics with the goal of achieving an opioid-sparing effect in the postoperative period.[14] A drug that can be safely used in the perianesthesia period and potentially demonstrate an opioid-sparing effect can be of exceptional use for an anesthesiologist.

Transient receptor potential vanilloid 1 (TRPV1) is an ion channel expressed predominantly in pain-sensing neurons.[15] This channel is activated by endogenous lipids, low pH, and temperature above 42°C, among other ligands.[16] Tonic activation of TRPV1 by low pH in the abdomen affects thermoeffectors and suppresses deep body temperature.[17–19] When this activation is blocked by TRPV1 antagonists, these drugs produce hyperthermia along with the desired effect of analgesia.[16–18] Additional advancement of TRPV1 antagonists in large clinical trials was halted due to the side effect of hyperthermia,[20] although some studies demonstrated analgesia.[21]

In this study, we tested the hypothesis that TRPV1 antagonists given on anesthesia induction prevent the development of anesthesia-induced hypothermia and decrease the opioid dose requirement for the reduction of postincision hyperalgesia. We tested our hypothesis in a rodent model of anesthesia-induced hypothermia and postsurgical hyperalgesia.

Materials and Methods

Animals

For experiments performed at the University of Arizona, the guidelines of University of Arizona (Tucson, Arizona) Institutional Animal Use and Care Committee (IACUC) were followed and the University of Arizona IACUC approved all of the animal protocols. The experiments performed at the University of Pecs (Pecs, Hungary) were conducted under protocols approved by the IACUC of the University of Pecs and conformed to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

Adult, male Sprague–Dawley rats (Envigo, Indianapolis, Indiana) were used and group housed in a temperature- and humidity-controlled room on a 12-h light/dark cycle. The adult rats had access to standard chow and water *ad libitum*, and the neonates were housed with the dam. Adult rats weighed approximately 250 to 300 g (University of Arizona experiments) or 519 ± 8 g (University of Pecs). Mice of both sexes had the Trpv1 gene either present (*Trpv1*$^{+/+}$) or missing (*Trpv1*$^{-/-}$), weighed approximately 19 g, and they were bred as described in details elsewhere.[22] All of the experiments were conducted in age- and body weight–matched animal groups.

Experimental Protocols

The experimenter was blinded to treatment allocations while giving the injection of vehicle or AMG 517 and also while performing temperature measurements and behavioral testing. The experimenter did not know whether the animals received vehicle or AMG 517 during the testing, and blinding was revealed at the end of the experiments. No randomization methods were used to assign animals to condition.

Anesthesia-induced Hypothermia

The experiments were performed at ambient room temperature of 22°C. Before anesthetic induction, baseline core body temperature was recorded using a wireless temperature probe (microthermia 2, ThermoWorks, Alpine, Utah) inserted at least one inch beyond the anal sphincter in gently restrained rats. Rats were lightly restrained and exposed to 5% isoflurane (Drager vaporizer, Drager, Telford, Pennsylvania) in 2 L oxygen until visible movement stopped and were maintained at 2.5% isoflurane in 2 L oxygen. Alternatively, lightly restrained rats were injected with intraperitoneal ketamine (100 mg/kg; Western Medical, Acadia, California). The tail vein was accessed and vehicle (10% dimethyl sulfoxide, 10% Tween 80, and 80% saline), AMG 517, or ABT-102 was administered as a bolus. Core body temperature was recorded every 5 min until the end of the experiment. ABT-102 (Axon Medchem, Reston, Virginia) and AMG 517 (Selleckchem, Boston, Massachusetts) were dissolved in 10% dimethyl sulfoxide, 10% Tween 80, and 80% saline (all from Sigma-Aldrich, St. Louis, Missouri) before the tail vein injection. All of the experiments were conducted in age- and body weight–matched animal groups.

Thermoregulation Experiments

These experiments were performed at the University of Pecs.

Mice. Similar to previously published studies,[23] each mouse was implanted with an intraperitoneal catheter, which allowed for nonstressful administration of the drugs during the experiment. Mice were anesthetized with an intraperitoneal injection of a ketamine–xylazine mixture (81.7 and 9.3 mg/kg, respectively) and received gentamycin (6 mg/kg subcutaneously) as an antibiotic prophylaxis. Experiments were performed 3 to 7 days after the surgery. During surgery, the mouse was placed on a heating pad (model TMP-5a; Supertech Instruments UK Ltd., London, United

Kingdom). After a small midline incision, a polyethylene (PE)-50 catheter filled with pyrogen-free saline was inserted into the peritoneal cavity. The internal end of the catheter was fixed to the left side of the abdominal wall with a suture; the free end of the catheter was tunneled under the skin to the nape, where it was exteriorized and heat sealed. The surgical abdominal wound was sutured in layers. The catheter was flushed with saline on the day after the surgery and every other day thereafter. On the day of the experiment, the mouse was placed in a cylindrical confiner and equipped with copper–constantan thermocouples (Omega Engineering, Stamford, Connecticut) to measure colonic temperature (a form of core body temperature) and tail skin temperature. The colonic thermocouple was inserted 3 cm deep beyond the anal sphincter and was fixed to the base of the tail with a loop of adhesive tape. The skin thermocouple was positioned on the lateral surface of tail at the border between the proximal and middle third of the tail and secured in placed with tape. The thermocouples were plugged into a datalogger device (Cole-Palmer, Vernon Hills, Illinois) connected to a computer. Mice in their confiners were then placed into a biochemistry incubator (model BJPX-Newark; Biobase, Jinan, China) set to an ambient temperature of 25°C, which is subneutral for mice in this setup. The intraperitoneal catheter was connected to a PE-50 extension filled with the drug of interest. The extension was passed through a port of the incubator and connected to a syringe.

Rats. As described before,[24] a rat designated to an experiment at the University of Pecs was implanted with an intraperitoneal and an intravenous (iv) catheter in the same surgery. The rat was anesthetized by ketamine–xylazine (55.6 and 5.5, respectively, intraperitoneally) and received an antibiotic (gentamycin, 6 mg/kg, subcutaneously) prophylactically. Experiments were performed 3 to 9 days after the surgery. After a small midline incision, a PE-50 catheter was inserted in the abdominal cavity. Similar as in mice, the internal end was fixed to the abdominal wall and the free end of the catheter was tunneled under the skin and exteriorized and sealed at the nape. The surgical wound was sutured in layers. For iv catheter implantation, a small longitudinal incision was made on the ventral surface of the neck, left of the trachea. The left jugular vein was exposed, freed from its surrounding connective tissue, and ligated. A silicone catheter (ID = 0.5 mm, OD = 0.9 mm) filled with heparinized (10 U/ml) saline was passed into the superior vena cava through the jugular vein and secured in place with ligatures. The free end of the catheter was knotted, tunneled under the skin to the nape, and exteriorized. The wound was sutured. Both the intraperitoneal and iv catheters were flushed on the day after the surgery and every other day. On the day of an experiment, the rats equipped with copper–constantan thermocouples (Omega Engineering) were placed in a confiner. The colonic thermocouple was inserted 10 cm beyond the anal sphincter. The skin thermocouple was positioned and fixed to the tail similarly as in mice. The thermocouples were plugged into a data logger (Cole-Parmer). Then, each rat in its confiner was transferred to a Plexiglas chamber of the four-chamber, open-circuit calorimeter integrated system (Oxymax Equal Flow, Columbus Instruments, Columbus, Ohio). The chamber was sealed, submerged into a temperature-controlled water bath, and continuously ventilated with room air (1,000 ml/min). The fractional concentration of oxygen was measured in the air entering and exiting the chamber, and the rate of oxygen consumption ($\dot{V}_{O_2}$) was calculated according to the manufacturer's instructions using the Oxymax Windows software (version 3.1, Columbus Instruments). The measurement of Vs $\dot{V}_{O_2}$ is a standard method used to assess the thermogenesis of rodents.[18,19,24–26] The intraperitoneal and iv catheters were connected to PE-50 extensions filled with the drug of interest. Each extension was passed through a port of the chamber and connected to a syringe, which was placed in a syringe pump (model 975; Harvard Apparatus Inc., Holliston, Massachusetts) in case of the iv extension. All of the experiments were conducted at an ambient temperature of 23°C, which is subneutral for rats in this setup.

Incisional Hyperalgesia Model, Measurement of Thermal and Mechanical Allodynia

Baseline thermal and mechanical withdrawal latencies were obtained using Hargreaves apparatus[27] and von Frey filaments, as described previously,[28] at least 24 h before the surgery. The animals were anesthetized using isoflurane as described above and treated with either vehicle or AMG 517 *via* the tail vein injection immediately on induction and just before performing surgery for incisional hyperalgesia, as described by Brennan *et al.*[29] The animals were maintained under isoflurane anesthesia (2.5%) until the surgery was performed (within 20 min) and allowed to recover from anesthesia.

Assessment of thermal and mechanical hyperalgesia was performed at 24 h postsurgery and was followed by the evaluation of the dose–response to morphine (National Institute of Drug Abuse, injected subcutaneously in the back) of rats that received preincision TRPV1 antagonist or vehicle treatment. The effect of morphine on postsurgical hyperalgesia was assessed 30, 60, and 120 min postmorphine injection.

Data Processing and Analysis

Sample sizes were determined based on our previous experience. Data on core body temperature, tail skin temperature, $\dot{V}_{O_2}$, and thermal and mechanical withdrawal latencies were compared by two-way ANOVA, repeated measures followed by Bonferroni *post hoc* tests, as appropriate. In ANOVA, we selected treatment (TRPV1 antagonist or vehicle as a between-subjects factor) and time (as a repeated-measure factor) as independent variables, although the dependent variables were core body temperature, tail skin temperature, $\dot{V}_{O_2}$, and thermal and mechanical withdrawal latencies. The criteria for statistical significance was $P < 0.05$, and the nature of

hypothesis testing was two tailed. For statistical analysis, Sigmaplot 11.0 (Systat Software, San Jose, California) software was used. All of the data are reported as mean ± SD.

Results

TRPV1 Antagonists Prevent General Anesthesia-induced Hypothermia

We first evaluated whether TRPV1 antagonists could prevent both volatile and intravenous anesthesia-induced hypothermia. We chose isoflurane and ketamine as our prototypical agents for volatile and intravenous anesthetics for various reasons. First, both of these agents have been well characterized in both humans and rodents with regard to their dose response and effect on thermoregulation. Second, respirometry chamber setup requires delivery of an anesthetic that is long lasting without an infusion or a vaporizer to correctly measure metabolic changes. A single intraperitoneal ketamine dose allowed us to perform these studies. We used two chemically different TRPV1 antagonists (AMG 517 and ABT-102). Importantly, both of these antagonists have already been shown in rodents and humans to induce hyperthermia in nonanesthetized subjects.[26,30] After obtaining baseline core body temperature, rats were anesthetized either using ketamine (fig. 1A) or isoflurane (5.0% induction, 2.5% maintenance in 2 L O_2; fig. 1B) and were injected with either vehicle or graded doses of intravenous AMG 517 (0.01 mg to 1 mg/kg) or with ABT-102 at 10 mg/kg. The doses of AMG 517 were chosen based on previous published studies.[26] As expected, both ketamine and isoflurane resulted in a significant decrease in the rats' core body temperature, with an average decrease of approximately 1.5°C (fig. 1, A through C), as is observed in humans. AMG 517 dose dependently prevented either ketamine- or isoflurane-induced hypothermia (fig. 1, A and B and Supplemental Digital Content 1, http://links.lww.com/ALN/B526, for scatterplot). Importantly, AMG 517 did not cause hyperthermia while the animals were maintained under anesthesia (fig. 1, A and B). The effect was statistically significant ($P < 0.001$, two way ANOVA, Bonferroni *post hoc* test) at all of the doses and time points in the ketamine experiments and at doses 0.1 and 1.0 mg/kg in the isoflurane experiments. To rule out the possibility that this effect was restricted to the particular pharmacophore of AMG 517, we used a completely unrelated drug, ABT-102, at a dose shown previously to cause hyperthermia[31] to prevent anesthesia-induced hypothermia. ABT-102 significantly ($P < 0.001$) prevented isoflurane-induced hypothermia at all of the time points tested (fig. 1C).

AMG 517 Does Not Prevent Anesthesia-induced Hypothermia in the Absence of TRPV1 Channels

Next, we studied whether AMG 517 prevention of anesthesia-induced hypothermia was specific to the blockade of TRPV1. After the induction of anesthesia with ketamine, *Trpv1*$^{+/+}$ and *Trpv1*$^{-/-}$ mice were infused with AMG 517 (1 mg/kg) or its

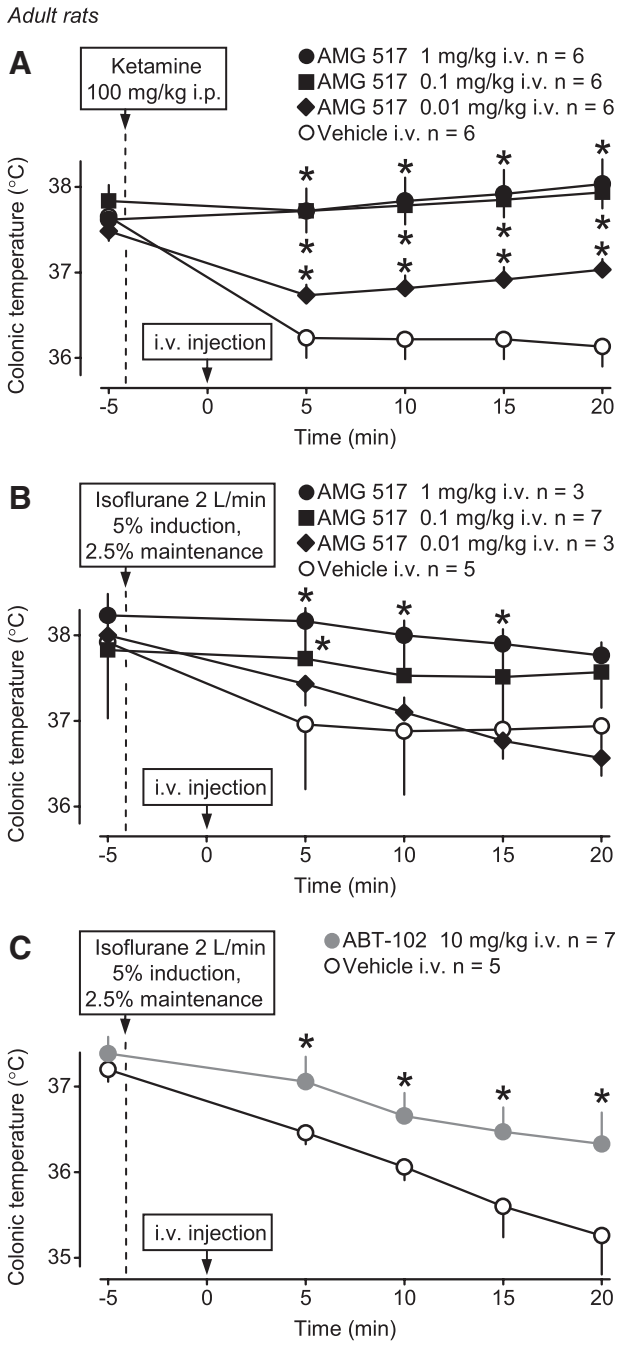

Fig. 1. Transient receptor potential vanilloid 1 (TRPV1) antagonists prevent general anesthesia-induced hyperthermia. (*A*) After acclimatizing to the testing chamber and obtaining baseline core body temperature, adult rats were injected with ketamine (100 mg/kg, intraperitoneally; i.p.). After the animals were anesthetized, either vehicle or AMG 517 (0.01 to 1.00 mg/kg) was injected in the tail vein and the core body temperature was measured every 5 min for 20 min. The AMG 517 prevention of ketamine-induced hypothermia was statistically significant at all doses and time points, and doses 0.1 to 1.0 mg/kg did not differ from baseline core body temperature (n = 6; *$P < 0.001$, two way ANOVA with Bonferroni *post hoc* test; *error bars* = SD). (*B*) In a similar setup as described above, adult rats were anesthetized with isoflurane

(Continued)

Fig. 1. (*Continued*) (2 L/min, 5.0% induction, 2.5% maintenance), and the anesthetized animals were injected with vehicle or AMG 517 (0.01 to 1 mg/kg intravenously; i.v.). The AMG 517 prevention of isoflurane-induced hypothermia was significant at 0.01 and 1.00 mg/kg at all of the time points tested (n = 3, 7, 3, 7; **P* < 0.05 two way ANOVA with Bonferroni *post hoc* test; *error bars* = SD). (*C*) In a similar setup as (*B*), after isoflurane-induced anesthesia, animals were treated with vehicle or ABT-102 (10 mg/kg iv). ABT-102 prevention of isoflurane-induced hypothermia was significant at all of the time points tested (n = 5 for vehicle; n = 7 for ABT-102; **P* < 0.001, two-way ANOVA with Bonferroni *post hoc* test; *error bars* = SD).

vehicle in a nonstressful manner, *via* a preimplanted catheter. To avoid technical problems associated with the catheterization of small murine veins, the catheter was implanted in the peritoneal cavity. Because mice are more sensitive to anesthetics than rats, ketamine was injected at 50 mg/kg intraperitoneally in these experiments. In *Trpv1*$^{+/+}$ mice, ketamine caused hypothermia with a nadir of 35.2° ± 0.4°C ($P < 0.001$) at 20 min after injection (fig. 2A). The core body temperature of the mice remained significantly ($P = 0.005$) lower than the baseline, even at 240 min after ketamine injection in the vehicle-treated group. When the *Trpv1*$^{+/+}$ mice were treated with AMG 517 instead of the vehicle, the ketamine-induced hypothermia was prevented at 40 min postinjection, and from that point the core body temperature of the mice did not decrease below the baseline throughout the experiment. The core body temperature of the AMG 517-treated *Trpv1*$^{+/+}$ mice was significantly ($P < 0.05$) higher than that of controls from 40 to 240 min after injection of ketamine. In *Trpv1*$^{-/-}$ mice, ketamine caused marked hypothermia between 10 and 240 min postinjection with similar dynamics as observed in the vehicle-treated *Trpv1*$^{+/+}$ mice. In contrast with the *Trpv1*$^{+/+}$ mice, the administration of AMG 517 did not prevent the ketamine-induced hypothermia in *Trpv1*$^{-/-}$ mice (fig. 2B). There was no significant difference between the core body temperature of AMG 517-treated and vehicle-treated *Trpv1*$^{-/-}$ mice throughout the experiment.

AMG 517 Prevents Ketamine-induced Hypothermia by Increasing Thermogenesis

We investigated which autonomic thermoeffectors contribute to the effect of AMG 517 on anesthesia-induced hypothermia. First, we administered AMG 517 or its vehicle after intraperitoneal injection of saline to confirm that AMG 517 causes hyperthermia in rats (fig. 3A). The administration of AMG 517 caused a pronounced increase of core body temperature with a peak of 38.9° ± 0.2°C at 80 min postinjection ($P < 0.001$). The core body temperature of the AMG 517–treated rats was significantly higher than that of controls between 30 and 240 min. The rise of core body temperature was accompanied by elevated $\dot{V}O_2$ in the AMG 517–treated animals, which was increased compared with controls from 30 to 190 min postinjection. Although an elevated $\dot{V}O_2$ can theoretically result from both shivering and nonshivering (brown fat) thermogenesis, in rats nonshivering

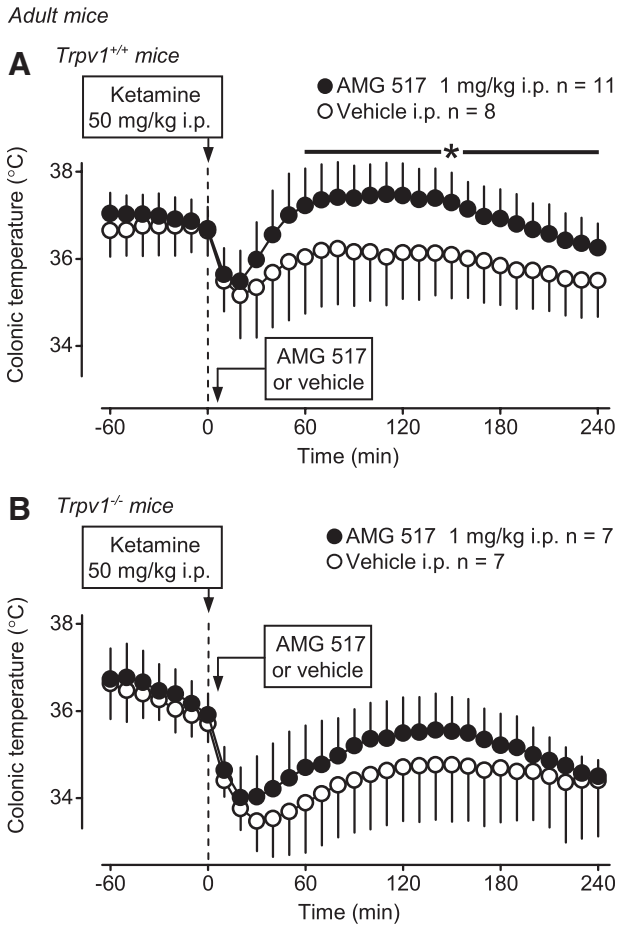

Fig. 2. AMG 517 does not prevent anesthesia-induced hypothermia in the absence of transient receptor potential vanilloid 1 (TRPV1) channels. (*A*) In wild-type (*Trpv1*$^{+/+}$) mice, ketamine (50 mg/kg intraperitoneally; i.p.) induced hypothermia that began within 20 min after the i.p. injection that sustained until the last measurement (240 min). However, when these animals were treated with AMG 517 (1 mg/kg i.p.), the ketamine-induced hypothermia was significantly prevented, starting at 40 min that persisted until the last measurement of 240 min (n = 8 for vehicle; n = 11 for AMG 517; **P* < 0.05, two-way ANOVA with Bonferroni *post hoc* test; *error bars* = SD). (*B*) In a setup similar to that used in (*A*), although ketamine (50 mg/kg i.p.) induced hypothermia in TRPV1 knockout (*Trpv1*$^{-/-}$) mice, AMG 517 (1 mg/kg i.p.) did not prevent ketamine-induced hypothermia at all of the time points tested (n = 7, two-way ANOVA showed no significant difference between the groups; *error bars* = SD).

thermogenesis is of greater importance.[32] These experiments were performed at a low ambient temperature of 23°C, which was needed for the development of anesthesia-induced hypothermia. In this subneutral environment, the tail skin temperature of the rats remained continuously low because of cold-induced vasoconstriction in both treatment groups. The effects of AMG 517 on core body temperature and autonomic thermoeffectors (fig. 3A) are in agreement with our previous results of the mechanisms of TRPV1 antagonist-induced hyperthermia.[18,19]

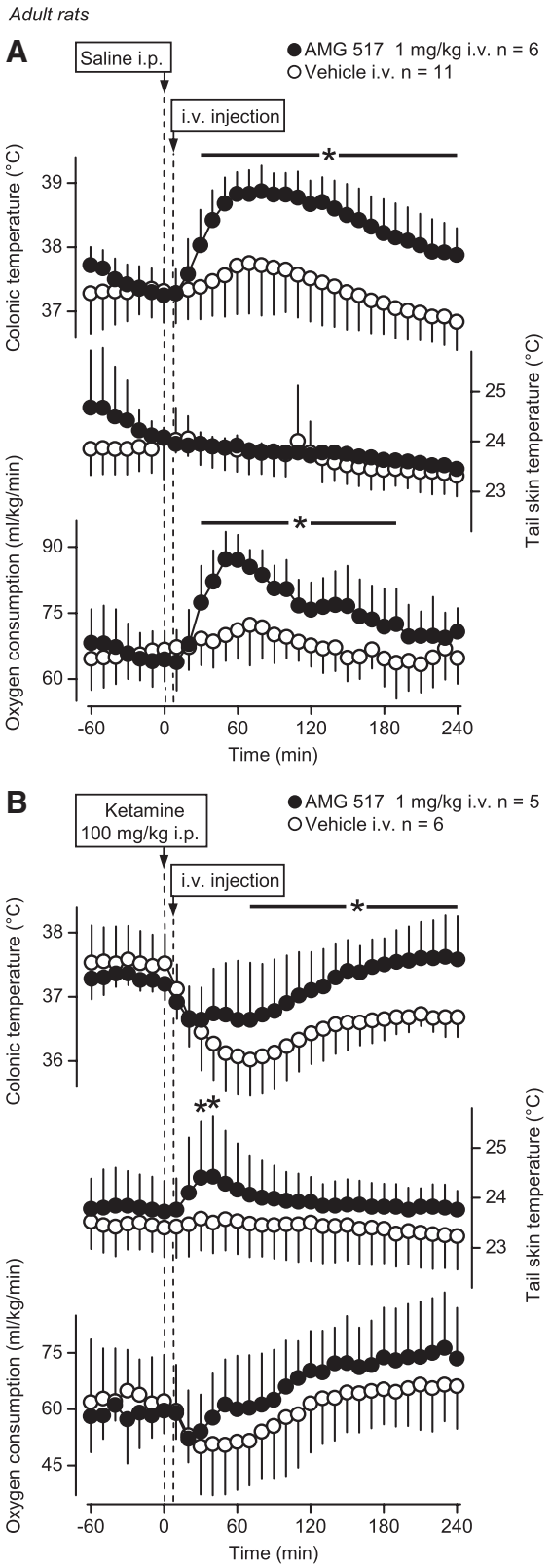

Fig. 3. AMG 517 prevents ketamine-induced hypothermia by increasing thermogenesis. (*A*) In unanesthetized rats (received saline intraperitoneally; i.p.), AMG 517 (1 mg/kg intravenously; i.v.) was injected, and changes in core body temperature,

(Continued)

Fig. 3. (*Continued*) tail skin temperature, and oxygen consumption ($\dot{V}O_2$) were measured.AMG 517 resulted in a statistically significant rise in core body temperature immediately after injection that lasted until the last measurement of 240 min (*top graph*). AMG 517 did not result in any significant change in tail skin temperature (*middle graph*) but significantly increased oxygen consumption (*bottom graph*) that was statistically significant from 30 to 190 min compared with the controls (n = 11 for vehicle; n = 6 for AMG 517; **P* < 0.05, two way ANOVA with Bonferroni *post hoc* test, *error bars* = SD). (*B*) In anesthetized rats (ketamine 100 mg/mg i.p.), AMG 517 (1 mg/kg iv) reversed ketamine-induced hypothermia (*top graph*) and transiently increased tail skin temperature (only until 40 min postinjection; *middle graph*). Ketamine (100 mg/kg) initially (20 to 100 min) decreased oxygen consumption ($\dot{V}O_2$; *bottom graph*) that was not observed in AMG 517–treated animals. Moreover, in AMG 517–treated animals, $\dot{V}O_2$ remained significantly higher at most time points (40 min onwards) compared with vehicle-treated animals (n = 6 vehicle; n = 5 AMG; **P* < 0.05, two-way ANOVA with Bonferroni *post hoc* test, ANOVA showed that the effect of AMG 517 on oxygen consumption was significant, $F(1, 9) = 11.083$; $P < 0.001$; *error bars* = SD).

In ketamine-treated rats, the iv administration of AMG 517 prevented the anesthesia-induced hypothermia (fig. 3B) in accordance with our findings described earlier (fig. 1A). The core body temperature of the AMG 517–treated rats was significantly higher than that of the controls from 70 to 240 min after drug administration. Regarding the thermoeffector pattern, we found that the ketamine-induced decrease of $\dot{V}O_2$ was markedly ($P < 0.001$) blunted after AMG 517 administration as compared with vehicle-treated rats. Interestingly, we recoded a transient yet significant increase in the tail skin temperature of AMG 517–treated rats compared with controls. The dynamics of the core body temperature response to ketamine were somewhat different in *Trpv1*$^{-/-}$ mice compared with their *Trpv1*$^{+/+}$ littermates. This was presumably caused by the characteristic thermoeffector pattern (*i.e.* enhanced vasoconstriction, hypometabolism, and hyperactivity), which is used by *Trpv1*$^{-/-}$ mice to regulate their core body temperature[23] and can be differently influenced by ketamine.

AMG 517 Prevents Ketamine and Isoflurane-induced Hypothermia without Causing Hyperthermia

TRPV1 antagonists cause hyperthermia in unanesthetized rodents and humans (for a review, see Romanovsky *et al.*[17]). Therefore, it was important to evaluate whether TRPV1 antagonists administered while the animals were anesthetized cause hyperthermia in the postanesthesia period. We addressed this question in both ketamine and isoflurane-induced anesthesia conditions. First, we administered AMG 517 (or its vehicle) to ketamine-treated rats and recorded their core body temperature for an extended, that is, 480-min, time period (fig. 4A). Long-term experiments can be exhaustive for the rats, thus, to reduce the potential distress of the experimental animals, we randomly selected four animals per group only for these experiments. Animals

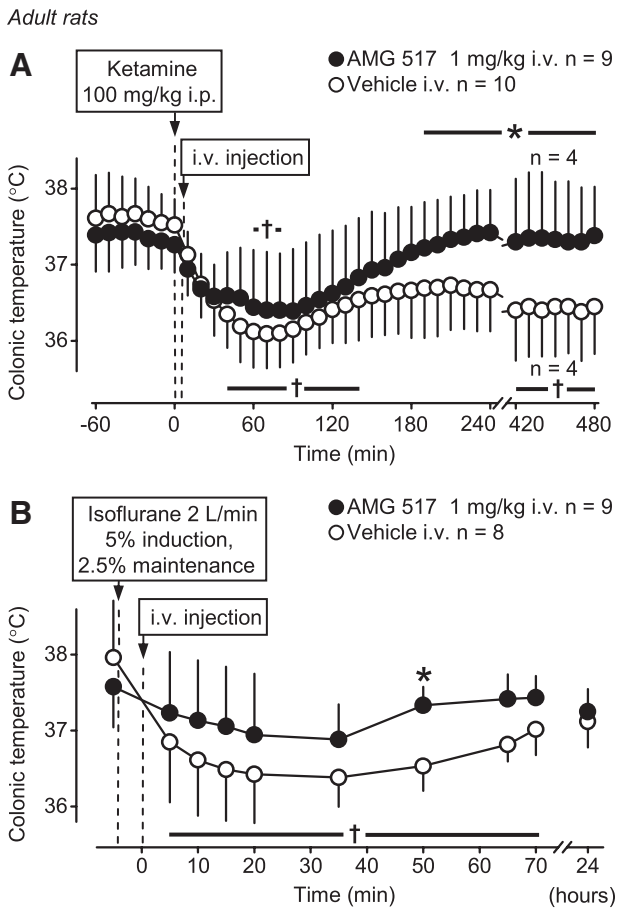

Fig. 4. AMG 517 prevents ketamine- and isoflurane-induced hypothermia without causing hyperthermia. (*A*) In a setup similar to that in figure 1A, the core body temperature of some of the animals (n = 4 each) were measured for a prolonged period of 480 min postketamine (100 mg/kg, intraperitoneally; i.p.) injection. Even after recovery from anesthesia (approximately 60 min postinjection), AMG517 did not cause hyperthermia in the postanesthesia period (*$P < 0.05$ *vs.* vehicle, †$P < 0.05$ *vs.* baseline; two-way ANOVA with Bonferroni *post hoc* test; *error bars* = SD). (*B*) In a setup similar to that in figure 1B, the core body temperature of the animals (n = 8 for vehicle; n = 9 for AMG 517) were measured for 50 min after isoflurane was turned off (20 min postinduction). When animals recovered from isoflurane anesthesia (15 min after gas was turned off), AMG 517 did not cause hyperthermia in any of the animals in the postanesthesia period (*$P < 0.05$ *vs.* vehicle, †$P < 0.05$ *vs.* baseline; two-way ANOVA with Bonferroni *post hoc* test; *error bars* = SD). i.v. = intravenously.

would typically recover from ketamine–anesthesia approximately 60 min postinjection. We found that the infusion of AMG 517 prevented the ketamine-induced hypothermia, as expected from our previous results in the current study (figs. 1A and 3B). Importantly, however, the core body temperature of the AMG 517–treated rats did not significantly differ from their baseline core body temperature even at 480 min postinjection (fig. 4A and Supplemental Digital Content 2, http://links.lww.com/ALN/B526). Similarly, after maintaining animals under isoflurane anesthesia for 20 min, the gas was turned off and the animals recovered from anesthesia in the next 15 min with their core body temperature approaching back to baseline at 50 min postcessation of anesthesia (fig. 4B). Importantly, while AMG 517 prevented isoflurane-induced hypothermia, it did not result in hyperthermia after anesthesia was terminated (20 to 70 min, as well as 24 h after AMG 517 injection; fig. 4B).

AMG 517 Pretreatment Decreases Opioid Dose Requirement for Reduction of Postincision Hyperalgesia

TRPV1 antagonists were primarily developed as analgesics. However, due to the unwanted side effect of hyperthermia, it is unlikely that they would be used in the postoperative period when the patient is not under anesthesia. Hence, we tested the hypothesis that TRPV1 antagonists given at anesthesia induction, before the incision is performed, decrease the opioid dose requirement for the reduction of postincision hyperalgesia. Both vehicle and AMG 517–pretreated animals developed thermal and mechanical hyperalgesia 24 h after incisional surgery (fig. 5). The extent of hyperalgesia development was not different in both groups. When we analyzed the antihyperalgesic response of morphine by comparing paw withdrawal latencies at various time points postmorphine injection, the morphine antihyperalgesia was significantly greater at the 0.3 and 0.6 mg/kg doses for thermal hypersensitivity (two-way repeated-measures ANOVA, Bonferroni *post hoc* test) in the AMG 517–treated animals in comparison with vehicle-treated animals (fig. 5A). Similarly, morphine reduction of incision-induced mechanical hyperalgesia was apparent at a lower dose of 0.3 mg/kg in AMG 517–treated animals but not in vehicle-treated animals (fig. 5B).

Discussion

We tested the hypothesis that TRPV1 antagonism prevents anesthesia-induced hypothermia. In addition, we determined that an additional potential benefit of the use of preincision TRPV1 antagonists may be to decrease opioid dosing required to reduce postincisional hypersensitivity. We found that two structurally distinct TRPV1 antagonists were able to prevent anesthesia-induced hypothermia after injected or volatile-induced general anesthesia. Importantly, TRPV1 antagonists did not cause hyperthermia in the setting of general anesthesia. Our data demonstrate that the prevention of anesthesia-induced hypothermia is specific to TRPV1 blockade, because it was absent in TRPV1 knockout mice. TRPV1 antagonists likely prevent anesthesia-induced hypothermia primarily by acting on brown fat thermogenesis. Finally, preincision TRPV1 antagonist treatment resulted in a decrease in the postincision morphine dose for antihyperalgesic effects in incisional hypersensitivity. These preclinical data suggest that TRPV1 antagonists may be useful in the perioperative phase, both to maintain normothermia and to reduce the requirements for opioid treatment of postoperative hyperalgesia.

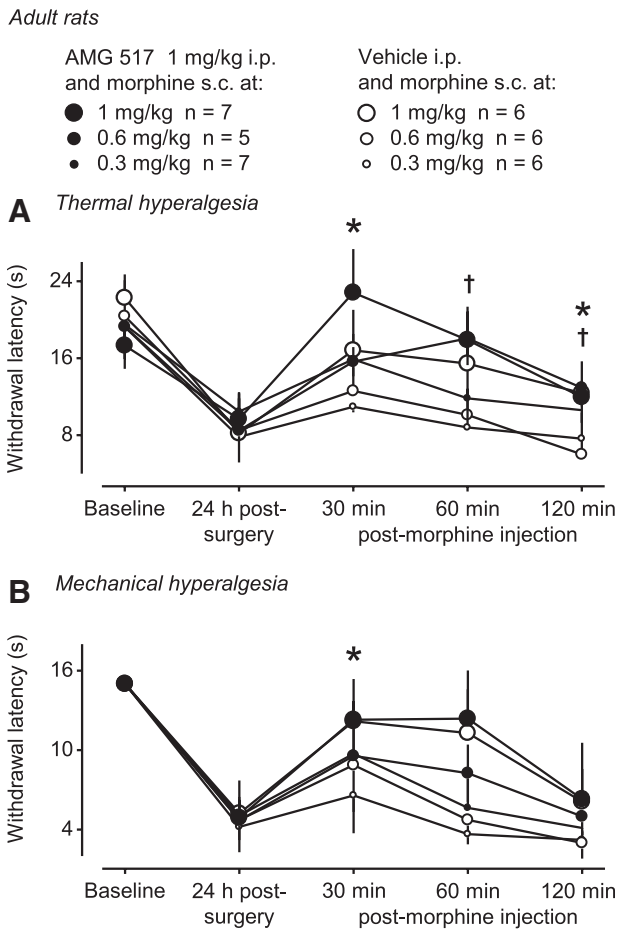

Fig. 5. AMG 517 pretreatment decreases opioid dose requirement for reduction of postincision hyperalgesia. (*A*) After obtaining baseline thermal withdrawal thresholds, rats were anesthetized with isoflurane as described above, treated with either vehicle or AMG 517 (1 mg/kg intravenously) before performing the hind paw surgery. The thermal withdrawal latencies were measured again 24 h postsurgery and then again 30 to 120 min postmorphine injection (0.3 to 1.0 mg/kg subcutaneously; s.c.). In AMG 517–pretreated rats, morphine significantly reduced thermal hyperalgesia at 0.3 and 0.6 mg/kg dose, whereas the same dose was ineffective in vehicle-pretreated rats (n = 6 for vehicle and n = 7, 5, 7 for AMG 517; **P* and †*P* < 0.05 *vs.* vehicle for morphine at 0.3 and 0.6 mg/kg, respectively; two-way ANOVA with Bonferroni *post hoc* test; *error bars* = SD). (*B*) In a similar paradigm as above, response to morphine on postsurgical mechanical withdrawal latencies was observed in vehicle or AMG 517–pretreated rats. In AMG 517–pretreated rats, morphine significantly reduced mechanical hyperalgesia at 0.3 mg/kg, whereas the same dose was ineffective in vehicle-pretreated rats (n = 6 for vehicle; n = 7, 5, 7 for AMG; **P* < 0.05 *vs*. vehicle for morphine at 0.3 mg/kg; two-way ANOVA with Bonferroni *post hoc* test; *error bars* = SD). i.p. = intraperitoneally.

After the molecular identification and characterization of the TRPV1 ion channel, antagonists were developed as potential nonopioid analgesics. However, this class of molecules was associated with the side effect of hyperthermia in both animals and in humans halting additional development for the treatment of pain.[17] Although hyperthermia is a major side effect, we hypothesized that, in the controlled setting of anesthesia in the operating room, this side effect could be exploited for therapeutic effect to pharmacologically counter anesthetic-induced hypothermia. AMG 517 and ABT-102 are structurally distinct TRPV1 antagonists that have been demonstrated to produce hyperthermia in rodents and humans.[16,31] Both compounds prevented anesthesia-induced hypothermia. The use of two structurally unrelated TRPV1 antagonists suggests a specific interaction of the molecules with the TRPV1 channel for prevention of anesthesia-induced hypothermia. This conclusion was further supported by the absence of the prevention of anesthesia-induced hypothermia in TRPV1 knockout mice.

Interestingly, despite its long half-life of approximately 20 h,[33] AMG 517 did not induce hyperthermia in the postanesthesia period at the doses used. Multiple possibilities could explain this paradoxical observation. First, the duration of AMG 517 thermoregulatory action may be different than its plasma half-life, as is seen with other drugs.[34,35] Second, general anesthetics inhibit autonomic cold-defense effectors (brown fat thermogenesis and cutaneous vasoconstriction),[36,37] an effect that may be counterbalanced by their possible disinhibition by TRPV1 antagonists. Finally, general anesthetics such as isoflurane have been demonstrated to activate TRPV1, which can desensitize TRPV1,[38] resulting in its inability to be further antagonized by AMG 517. If the lack of hyperthermic effect in the postanesthesia phase is also confirmed in humans, it may help mitigate one of the concerns regarding the potential use of TRPV1 antagonists in the perioperative phase.

We observed that anesthesia-induced hypothermia was not altered in TRPV1 knockout mice, suggesting that TRPV1 is not the primary mediator for anesthesia-induced hypothermia. Nevertheless, the effect of AMG 517 depended on the expression of TRPV1. Because AMG 517 also prevented the anesthesia-induced hypothermia in wild-type mice in addition to rats, our findings demonstrate that antagonism of TRPV1 for the prevention of anesthesia-induced hypothermia is not species specific. Previous studies have demonstrated that AMG 517 causes hyperthermia in both rodents and humans,[18,19,26,39] suggesting that its thermoregulatory effects are preserved in humans. Additional studies are needed to determine whether AMG 517 can prevent anesthesia-induced hypothermia in humans.

In the present studies, the TRPV1 antagonist was administered immediately after anesthesia induction to test whether TRPV1 antagonism can reverse mild hypothermia that occurs as soon as anesthesia is induced and prevent the development of additional hypothermia with ongoing anesthesia. A question that we did not directly address in this article is whether the treatment with the TRPV1 antagonist can reverse an already established hypothermic state. The experimental protocol used in these studies necessitated the induction of general anesthesia that was followed by the injection of the TRPV1 antagonist. As a result, almost all of the animals had mild hypothermia (approximately 0.25° to 0.50°C) before the

TRPV1 antagonist was administered. This also means that activation thresholds of the different thermoeffectors could already be shifted as a consequence of general anesthesia.[40] Under these conditions, TRPV1 antagonists were able maintain normothermia while the animals were under anesthesia.

In the experiments evaluating the mechanism behind TRPV1 antagonist prevention of anesthesia-induced hypothermia, we evaluated both vasomotor tone and oxygen consumption. In unanesthetized animals, brown fat thermogenesis and skin vasoconstriction were the mechanisms by which the TRPV1 antagonist induced hyperthermia.[18] In anesthetized animals as well, TRPV1 antagonist prevention of anesthesia-induced hypothermia was associated with increasing thermogenesis. We observed a transient increase in tail skin temperature, suggesting a change in vasomotor tone. However, this effect was neither pronounced nor long lasting and may have been brought about by a downward shift in the threshold deep body temperature for vasodilation by ketamine, which in itself was not enough to overcome the vasoconstrictor effect of the low ambient temperature used. However, AMG 517 disinhibits thermogenesis,[19,26] so the core body temperature of ketamine-anesthetized rats moved up compared with ketamine-anesthetized controls without AMG 517 and crossed the threshold deep body temperature for vasodilation.

TRPV1 antagonists were originally being developed as analgesics. Given the likely importance of TRPV1 in the development and maintenance of postsurgical pain, we studied the possible effect of preincision TRPV1 blockade on the development of postincision hyperalgesia and the dose of morphine required to reduce it. Preincision TRPV1 blockade did not prevent the development of postincision thermal and mechanical hyperalgesia (fig. 5). This finding is in agreement with previous studies demonstrating limited efficacy of TRPV1 antagonists alone in incisional pain.[41] However, this is in contrast with other studies that have demonstrated the key role of TRPV1 in the development of incisional thermal hyperalgesia using TRPV1 knockout animals.[42] It is possible that either higher or repeated preincision dosing of TRPV1 antagonists is required to replicate results obtained in knockout animals. Alternatively, preincision TRPV1 antagonists may reduce ongoing pain, which is not tested in these assays. Importantly, preincision TRPV1 blockade resulted in the appearance of opioid antihyperalgesia at lower doses. Multiple possible mechanisms might underlie this observation. First, preincision TRPV1 blockade may decrease central sensitization by blocking primary afferent barrage or central TRPV1 activation.[43,44] Second, TRPV1 antagonism may block acute opioid tolerance, because activation of TRPV1 has been implicated as one of the mechanisms underlying acute opioid tolerance and opioid-induced hyperalgesia.[45,46]

Future studies may help to determine whether advancement of TRPV1 antagonists to clinical practice is possible. After the discovery of hyperthermia as a major side effect of TRPV1 antagonists, multiple approaches were taken to separate the analgesic and hyperthermic effects of TRPV1 antagonists. One study compared different pharmacophores necessary for the hyperthermic *versus* analgesic effects.[19] Using a similar approach, future studies may focus on developing pharmacophores designed to enhance the antihypothermia effect while retaining the analgesia. The clinical consequences of anesthesia-induced hypothermia are on bleeding, postoperative myocardial infarction, and surgical site infection. Although conceivable, it would be important to study whether maintenance of normothermia by TRPV1 antagonists prevents these consequences. Moreover, TRPV1 antagonists also affect the mortality rate in murine models of systemic inflammation, both in age-specific and inflammation-specific (septic *vs*. aseptic) ways.[39,47] These findings suggest that the use of TRPV1 antagonists during surgery may have additional advantages.

In summary, this study demonstrates the possibility of a novel use of TRPV1 antagonists to prevent anesthesia-induced hypothermia while simultaneously decreasing postoperative opioid requirements. Because both drugs described here (AMG 517 and ABT-102) have already been advanced to human trials, this hypothesis can be evaluated in humans undergoing anesthesia.

Acknowledgments

The authors thank Jessica Hansen, B.S., Department of Pharmacology, University of Arizona, Tucson, Arizona, for her help conducting the experiments.

Research Support

Supported by a Foundation for Anesthesia Education and Research grant (Schaumburg, Illinois; to Dr. Patwardhan), a University of Arizona Career Development grant (Tucson, Arizona; to Dr. Patwardhan), and in part by the Research Fund of the Medical School, University of Pecs (Pecs, Hungary; grant No. KA-2016-15) and the New National Excellence Program of the Hungarian Ministry of Human Capacities (Budapest, Hungary; UNKP-16-4-III).

Competing Interests

Drs. Patwardhan and Porreca declare a financial interest in Catalina Pharmaceuticals (Tucson, Arizona), which licenses the intellectual property involved in this research. Drs. Patwardhan, Porreca, and Romanovsky are founders of Catalina Pharma Inc. (Tucson, Arizona) and hold a provisional patent for the use of transient receptor potential vanilloid 1 antagonists in the prevention of anesthesia-induced hypothermia. Dr. Romanovsky has consulted for Abbott Laboratories (Chicago, Illinois), AbbVie (Chicago, Illinois), Amgen Inc. (Thousand Oaks, California), Japan Tobacco Inc. (Geneva, Switzerland), Teva Pharmaceutical Industries Ltd. (North Wales, Pennsylvania), and other pharmaceutical companies, and his research has been supported by Abbott Laboratories, AbbVie, and Amgen Inc. The other authors declare no competing interests.

Correspondence

Address correspondence to Dr. Patwardhan: Departments of Anesthesiology and Pharmacology, University of Arizona,

1501 North Campbell Avenue, Tucson, Arizona 85724. apatwardhan@anesth.arizona.edu. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

1. Sessler DI: Mild perioperative hypothermia. N Engl J Med 1997; 336:1730–7
2. Butwick AJ, Lipman SS, Carvalho B: Intraoperative forced air-warming during cesarean delivery under spinal anesthesia does not prevent maternal hypothermia. Anesth Analg 2007; 105:1413–9
3. Lin EP, Smith K, Valley RD: Wet forced-air warming blankets are ineffective at maintaining normothermia. Paediatr Anaesth 2008; 18:642–4
4. Leben J, Tryba M: Prevention of hypothermia during surgery: Contribution of convective heating system and warm infusion. Ann N Y Acad Sci 1997; 813:807–11
5. Brandes IF, Jipp M, Popov AF, Seipelt R, Quintel M, Bräuer A: Intensified thermal management for patients undergoing transcatheter aortic valve implantation (TAVI). J Cardiothorac Surg 2011; 6:117
6. McGovern PD, Albrecht M, Belani KG, Nachtsheim C, Partington PF, Carluke I, Reed MR: Forced-air warming and ultra-clean ventilation do not mix: An investigation of theatre ventilation, patient warming and joint replacement infection in orthopaedics. J Bone Joint Surg Br 2011; 93:1537–44
7. Baker N, King D, Smith EG: Infection control hazards of intraoperative forced air warming. J Hosp Infect 2002; 51:153–4
8. Sessler DI, Olmsted RN, Kuelpmann R: Forced-air warming does not worsen air quality in laminar flow operating rooms. Anesth Analg 2011; 113:1416–21
9. Rathmell JP, Wu CL, Sinatra RS, Ballantyne JC, Ginsberg B, Gordon DB, Liu SS, Perkins FM, Reuben SS, Rosenquist RW, Viscusi ER: Acute post-surgical pain management: A critical appraisal of current practice, December 2-4, 2005. Reg Anesth Pain Med 2006; 31(4 suppl 1):1–42
10. Thomas T, Robinson C, Champion D, McKell M, Pell M: Prediction and assessment of the severity of post-operative pain and of satisfaction with management. Pain 1998; 75:177–85
11. Kehlet H, Jensen TS, Woolf CJ: Persistent postsurgical pain: Risk factors and prevention. Lancet 2006; 367:1618–25
12. Kehlet H, Holte K: Effect of postoperative analgesia on surgical outcome. Br J Anaesth 2001; 87:62–72
13. Souter AJ, Fredman B, White PF: Controversies in the perioperative use of nonsterodial antiinflammatory drugs. Anesth Analg 1994; 79:1178–90
14. Woolf CJ, Chong MS: Preemptive analgesia: Treating postoperative pain by preventing the establishment of central sensitization. Anesth Analg 1993; 77:362–79
15. Cavanaugh DJ, Chesler AT, Jackson AC, Sigal YM, Yamanaka H, Grant R, O'Donnell D, Nicoll RA, Shah NM, Julius D, Basbaum AI: Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. J Neurosci 2011; 31:5067–77
16. Gavva NR: Body-temperature maintenance as the predominant function of the vanilloid receptor TRPV1. Trends Pharmacol Sci 2008; 29:550–7
17. Romanovsky AA, Almeida MC, Garami A, Steiner AA, Norman MH, Morrison SF, Nakamura K, Burmeister JJ, Nucci TB: The transient receptor potential vanilloid-1 channel in thermoregulation: A thermosensor it is not. Pharmacol Rev 2009; 61:228–61
18. Steiner AA, Turek VF, Almeida MC, Burmeister JJ, Oliveira DL, Roberts JL, Bannon AW, Norman MH, Louis JC, Treanor JJ, Gavva NR, Romanovsky AA: Nonthermal activation of transient receptor potential vanilloid-1 channels in abdominal viscera tonically inhibits autonomic cold-defense effectors. J Neurosci 2007; 27:7459–68
19. Garami A, Shimansky YP, Pakai E, Oliveira DL, Gavva NR, Romanovsky AA: Contributions of different modes of TRPV1 activation to TRPV1 antagonist-induced hyperthermia. J Neurosci 2010; 30:1435–40
20. Wong GY, Gavva NR: Therapeutic potential of vanilloid receptor TRPV1 agonists and antagonists as analgesics: Recent advances and setbacks. Brain Res Rev 2009; 60:267–77
21. Quiding H, Jonzon B, Svensson O, Webster L, Reimfelt A, Karin A, Karlsten R, Segerdahl M: TRPV1 antagonistic analgesic effect: A randomized study of AZD1386 in pain after third molar extraction. Pain 2013; 154:808–12
22. Bölcskei K, Helyes Z, Szabó A, Sándor K, Elekes K, Németh J, Almási R, Pintér E, Petho G, Szolcsányi J: Investigation of the role of TRPV1 receptors in acute and chronic nociceptive processes using gene-deficient mice. Pain 2005; 117:368–76
23. Garami A, Pakai E, Oliveira DL, Steiner AA, Wanner SP, Almeida MC, Lesnikov VA, Gavva NR, Romanovsky AA: Thermoregulatory phenotype of the Trpv1 knockout mouse: Thermoeffector dysbalance with hyperkinesis. J Neurosci 2011; 31:1721–33
24. Banki E, Pakai E, Gaszner B, Zsiboras C, Czett A, Bhuddi PR, Hashimoto H, Toth G, Tamas A, Reglodi D, Garami A: Characterization of the thermoregulatory response to pituitary adenylate cyclase-activating polypeptide in rodents. J Mol Neurosci 2014; 54:543–54
25. de Oliveira C, Garami A, Lehto SG, Pakai E, Tekus V, Pohoczky K, Youngblood BD, Wang W, Kort ME, Kym PR, Pinter E, Gavva NR, Romanovsky AA: Transient receptor potential channel ankyrin-1 is not a cold sensor for autonomic thermoregulation in rodents. J Neurosci 2014; 34:4445–52
26. Gavva NR, Treanor JJ, Garami A, Fang L, Surapaneni S, Akrami A, Alvarez F, Bak A, Darling M, Gore A, Jang GR, Kesslak JP, Ni L, Norman MH, Palluconi G, Rose MJ, Salfi M, Tan E, Romanovsky AA, Banfield C, Davar G: Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. Pain 2008; 136:202–10
27. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988; 32:77–88
28. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL: Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994; 53:55–63
29. Brennan TJ, Vandermeulen EP, Gebhart GF: Characterization of a rat model of incisional pain. Pain 1996; 64:493–501
30. Othman AA, Nothaft W, Awni WM, Dutta S: Effects of the TRPV1 antagonist ABT-102 on body temperature in healthy volunteers: Pharmacokinetic/pharmacodynamic analysis of three phase 1 trials. Br J Clin Pharmacol 2013; 75:1029–40
31. Honore P, Chandran P, Hernandez G, Gauvin DM, Mikusa JP, Zhong C, Joshi SK, Ghilardi JR, Sevcik MA, Fryer RM, Segreti JA, Banfor PN, Marsh K, Neelands T, Bayburt E, Daanen JF, Gomtsyan A, Lee CH, Kort ME, Reilly RM, Surowy CS, Kym PR, Mantyh PW, Sullivan JP, Jarvis MF, Faltynek CR: Repeated dosing of ABT-102, a potent and selective TRPV1 antagonist, enhances TRPV1-mediated analgesic activity in rodents, but attenuates antagonist-induced hyperthermia. Pain 2009; 142:27–35
32. Cannon B, Nedergaard J: Brown adipose tissue: Function and physiological significance. Physiol Rev 2004; 84:277–359
33. Doherty EM, Fotsch C, Bannon AW, Bo Y, Chen N, Dominguez C, Falsey J, Gavva NR, Katon J, Nixey T, Ognyanov VI, Pettus L, Rzasa RM, Stec M, Surapaneni S, Tamir R, Zhu J, Treanor JJ, Norman MH: Novel vanilloid receptor-1 antagonists: 2–Structure-activity relationships of 4-oxopyrimidines leading to the selection of a clinical candidate. J Med Chem 2007; 50:3515–27

34. Ali-Melkkilä T, Kanto J, Iisalo E: Pharmacokinetics and related pharmacodynamics of anticholinergic drugs. Acta Anaesthesiol Scand 1993; 37:633–42
35. Herbette LG, Vecchiarelli M, Sartani A, Leonardi A: Lercanidipine: Short plasma half-life, long duration of action and high cholesterol tolerance–Updated molecular model to rationalize its pharmacokinetic properties. Blood Press Suppl 1998; 2:10–7
36. Ohlson KB, Lindahl SG, Cannon B, Nedergaard J: Thermogenesis inhibition in brown adipocytes is a specific property of volatile anesthetics. Anesthesiology 2003; 98:437–48
37. Sessler DI, McGuire J, Moayeri A, Hynson J: Isoflurane-induced vasodilation minimally increases cutaneous heat loss. Anesthesiology 1991; 74:226–32
38. Kimball C, Luo J, Yin S, Hu H, Dhaka A: The pore loop domain of TRPV1 is required for its activation by the volatile anesthetics chloroform and isoflurane. Mol Pharmacol 2015; 88:131–8
39. Wanner SP, Garami A, Pakai E, Oliveira DL, Gavva NR, Coimbra CC, Romanovsky AA: Aging reverses the role of the transient receptor potential vanilloid-1 channel in systemic inflammation from anti-inflammatory to proinflammatory. Cell Cycle 2012; 11:343–9
40. Sessler DI: Perioperative thermoregulation and heat balance. Lancet 2016; 387:2655–64
41. Wu C, Gavva NR, Brennan TJ: Effect of AMG0347, a transient receptor potential type V1 receptor antagonist, and morphine on pain behavior after plantar incision. Anesthesiology 2008; 108:1100–8
42. Pogatzki-Zahn EM, Shimizu I, Caterina M, Raja SN: Heat hyperalgesia after incision requires TRPV1 and is distinct from pure inflammatory pain. Pain 2005; 115:296–307
43. Planells-Cases R, Garcìa-Sanz N, Morenilla-Palao C, Ferrer-Montiel A: Functional aspects and mechanisms of TRPV1 involvement in neurogenic inflammation that leads to thermal hyperalgesia. Pflugers Arch 2005; 451:151–9
44. Patwardhan AM, Scotland PE, Akopian AN, Hargreaves KM: Activation of TRPV1 in the spinal cord by oxidized linoleic acid metabolites contributes to inflammatory hyperalgesia. Proc Natl Acad Sci U S A 2009; 106:18820–4
45. Vardanyan A, Wang R, Vanderah TW, Ossipov MH, Lai J, Porreca F, King T: TRPV1 receptor in expression of opioid-induced hyperalgesia. J Pain 2009; 10:243–52
46. Nguyen TL, Nam YS, Lee SY, Kim HC, Jang CG: Effects of capsazepine, a transient receptor potential vanilloid type 1 antagonist, on morphine-induced antinociception, tolerance, and dependence in mice. Br J Anaesth 2010; 105:668–74
47. Guptill V, Cui X, Khaibullina A, Keller JM, Spornick N, Mannes A, Iadarola M, Quezado ZM: Disruption of the transient receptor potential vanilloid 1 can affect survival, bacterial clearance, and cytokine gene expression during murine sepsis. Anesthesiology 2011; 114:1190–9