Transient Receptor Potential Ankyrin 1 Ion Channel Contributes to Guarding Pain and Mechanical Hypersensitivity in a Rat Model of Postoperative Pain

Hong Wei, Ph.D.,* Mari Karimaa, M.Sc.,† Timo Korjamo, Ph.D.,‡ Ari Koivisto, Ph.D.,‡ Antti Pertovaara, M.D., Ph.D.§

ABSTRACT

Background: The transient receptor potential ankyrin 1 (TRPA1) ion channel is expressed on nociceptive primary afferent nerve fibers. On the distal ending, it is involved in transduction of noxious stimuli, and on the proximal ending (within the spinal dorsal horn), it regulates transmission of nociceptive signals. Here we studied whether the cutaneous or spinal TRPA1 ion channel contributes to mechanical hypersensitivity or guarding, an index of spontaneous pain, in an experimental model of postoperative pain in the rat.

Methods: A skin plus deep-tissue incision was performed under general anesthesia in the plantar skin of one hind paw, after which the incised skin was closed with sutures. Postoperative pain and hypersensitivity were assessed 24–48 h after the operation. Guarding pain was assessed by scoring the hind-paw position. Mechanical hypersensitivity was assessed with a calibrated series of monofilaments applied to the wound area in the operated paw or the contralateral control paw. Chembridge-5861528, a TRPA1 channel antagonist, was administered intraperitoneally (10–30 mg/kg), intraplantarly (10–30 μg), or intrathecally (10 μg) in attempts to suppress guarding and hypersensitivity.

Results: Intraperitoneal or ipsilateral intraplantar treatment with Chembridge-5861528 reduced mechanical hypersensitivity and guarding in the operated limb. Intrathecal treatment attenuated hypersensitivity but not guarding. Intraplantar Chembridge-5861528 suppressed preferentially mechanical hyperalgesia and intrathecal Chembridge-5861528 tactile allodynia.

Conclusions: The TRPA1 channel in the skin contributes to sustained as well noxious mechanical stimulus-evoked postoperative pain, whereas the spinal TRPA1 channel contributes predominantly to innocuous mechanical stimulus-evoked postoperative pain.

What We Already Know about This Topic

• The transient receptor potential ankyrin-1 (TRPA1) ion channel is expressed on peripheral nerves and important to transduction and transmission of pain
• The role of TRPA1 ion channels in postoperative pain is unknown

What This Article Tells Us That Is New

• In rats, peripheral administration of a TRPA1 antagonist reduced hypersensitivity and pain behavior from paw incision, whereas spinal administration primarily reduced hypersensitivity to light touch
• TRPA1 ion channels represent an interesting target for treating postoperative pain

* Postdoctoral Fellow, § Professor of Physiology, Institute of Biomedicine/Physiology, University of Helsinki, Helsinki, Finland. † Research Scientist, ‡ Senior Research Scientist, Orion Corporation, OrionPharma, Turku, Finland.

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Address correspondence to Dr. Pertovaara: Institute of Biomedicine/Physiology, POB 63, University of Helsinki, FIN-00014 Helsinki, Finland. antti.pertovaara@helsinki.fi. 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.

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TRANSIENT receptor potential ankyrin 1 (TRPA1) is a nonselective cation channel expressed on nociceptive primary afferent nerve fibers. On peripheral terminals, it contributes to transduction of potentially harmful stimuli to nociceptive signals, and on central terminals in the spinal dorsal horn, it regulates glutamatergic transmission to spinal interneurons. Consequently, blocking the TRPA1 channel is expected to reduce nociception induced or enhanced by various TRPA1 channel agonists that include various irritant chemicals (such as cinnamaldehyde or reactive oxygen species) and noxious mechanical stimulation. In line with this, there is accumulating evidence indicating that TRPA1 channel antagonists or a knockout of the TRPA1 channel reduce pain behavior evoked by, e.g., mechanical stimulation or chemical compounds acting on the TRPA1 channel.

Following surgery, sustained pain and mechanical hypersensitivity in the operated region are common complications. It is not yet known whether the TRPA1 channel plays a role in mechanical hypersensitivity or sustained pain in postoperative conditions. An experimental animal model of postoperative pain allows assessing treatment effects both on mechanical hypersensitivity and an index of ongoing pain, guarding behavior. In the present study we determined whether blocking the TRPA1 channel with a selective antagonist attenuates postoperative hypersensitivity to mechanical stimulation, sustained postoperative pain behavior, or both following operation of the hind paw in the rat. To assess whether the TRPA1 channel expressed on the cutaneous ending of the nociceptive nerve fiber exerts a role different from that expressed by the TRPA1 channel on the central ending, postoperative guarding behavior and mechanical hypersensitivity were assessed following intraplantar and intrathecal as well as intraperitoneal administration of a selective TRPA1 channel antagonist. Moreover, to assess whether the potential contribution of the TRPA1 channel to postoperative hypersensitivity varies with the intensity of mechanical stimulation, the antihypersensitivity effect was assessed with mechanical test stimuli of innocuous and noxious intensity. Furthermore, in one group of animals, the TRPA1 channels in the operated skin area were blocked before operation to assess whether blocking the TRPA1 channel has a preemptive effect on postoperative pain and hypersensitivity.

Materials and Methods

Experimental Animals

The experiments were performed with male Hannover-Wistar rats (220–260 g; Scanbur Ab, Sollentuna, Sweden) in Biomedicum Helsinki, University of Helsinki, Finland. All experiments were approved by the ethical committee for experimental animal studies of the State Provincial Office of Southern Finland (Hämeenlinna, Finland), and the experiments were performed according to the guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available. The animals were housed in polycarbonate cages with a deep layer of sawdust, 1–3 animals in each cage, in a thermostatically controlled room at 24.0 ± 0.5°C. The room was artificially illuminated from 8:30 AM to 8:30 PM. The animals received commercial pelleted rat feed (CRM-P pellets; Special Diets Services, Witham, Essex, England) and tap water ad libitum.

TRPA1 Channel Antagonists

Chembridge-5861528, (CHEM; a TRPA1 channel antagonist and derivative of HC-030031) was synthesized by Chembridge Corporation (San Diego, CA). Calcium imaging results in human TRPA1 and transient receptor potential vanilloid 1 (TRPV1) transfected human embryonic kidney 293 (HEK) cells showed that when mustard oil or 4-hydroxynonenal (4-HNE) was used as a TRPA1 channel agonist, half-maximal inhibitory concentration (IC50) value of CHEM was 14.3 ± 0.7 μM or 18.7 ± 0.3 μM, respectively. Moreover, CHEM showed no TRPA1 or TRPV1 channel agonism and no TRPV1 channel antagonism up to a dose of 100 μM. Patch clamp recordings in rat TRPA1 transfected human embryonic kidney 293 cells indicated that CHEM is a reversible rat TRPA1 channel antagonist with IC50 of 230 nM and a Hill coefficient of 0.6. For intraperitoneal administrations, CHEM was dissolved in 0.5% methylcellulose. A-967079, a selective TRPA1 channel antagonist with a structure different from CHEM, was used for comparison.

Surgical Procedures for the Induction of Postoperative Pain and Installation of Intrathecal Catheter

For skin plus deep-tissue incision, the animals were anesthetized with sodium pentobarbitone (50 mg/kg intraperitoneally). If the level of anesthesia was not deep enough, as indicated by a movement response to noxious pinch of the skin or to skin surgery, additional doses of pentobarbitone (15–20 mg/kg intraperitoneally) were given as needed. However, because of short duration of the operation (fewer than 10 min), additional doses were not needed after induction of surgical level of anesthesia in any of the animals. Skin plus deep-tissue incision in the plantar skin of one hind paw was performed as described in detail earlier. Briefly, beginning 0.5 cm from the proximal edge of the heel, a 1-cm longitudinal incision was made through the skin and underlying fascia and the plantar flexor digitorum brevis muscle with a No. 11 surgical blade. Blunt curved forceps were then inserted through the incision into the muscle to further divide and retract the muscle. The muscle origin and insertion remained intact. The wound was then closed with three subcutaneous mattress sutures. After the operation, the animals were allowed to recover for 24 h in their home cages.

In a group of animals, drugs were administered intrathecally. For intrathecal drug injections, a catheter (Intramedic PE-10; Becton Dickinson and Company, Sparks, MD) was administered into the lumbar level of the spinal cord under pentobarbital anesthesia (50 mg/kg intraperitoneally) as de-
scribed in detail elsewhere. Following recovery from anesthesia, the correct placing of the catheter was verified by administering lidocaine (4%, 7–10 μl followed by 15 μl of saline for flushing) with a 50-μl Hamilton syringe (Hamilton Company, Bonaduz, Switzerland). Only the rats which had no motor impairment before lidocaine injection but had a bilateral paralysis of hind limbs following intrathecal administration of lidocaine were studied further. The installation of the intrathecal catheter was performed about 1 week before the start of the actual experiments.

**Assessment of Pain Behavior**

All animals were habituated to pain-testing procedures at least 1 or 2 h per day for 2 days before the surgery and before assessing drug effects on pain behavior. For assessment of guarding behavior, which was considered to represent ongoing postoperative pain, rats were placed individually on a grid covered with a clear plastic cage top. The hind paws were closely observed during a 10-min period starting 20 min after intraperitoneal injection of the studied compound. Since preliminary studies indicated that guarding was negligible in the control paw, guarding in the actual experiments was assessed only in the injured paw. According to the hind paw position, a score of 0, 1, or 2 was given. Zero was scored when the incised area was touching the mesh and the area was blanched or distorted by the mesh; 1 was scored when the incised area touched the mesh without blanching or distortion; and 2 was scored when the incised area was completely off of the mesh. For each score, the duration of behavior was measured during the 10-min observation period. Duration of guarding behavior was not significantly different between the first two postoperative days (PD1 and D2) that were used for drug testing in the present study (main effect of postoperative day: F1,32 = 0.64; see panel A in Supplemental Digital Content 1, http://links.lww.com/ALN/A856, which is a graph showing the durations of type 1 and type 2 guarding on the first two postoperative days). Duration of type 1 guarding was significantly longer than that of type 2 guarding (main effect of the type of guarding: F1,32 = 111.5, P < 0.0001), independent of the postoperative day (interaction between postoperative day and type of guarding: F1,32 = 0.19; see panel A in Supplemental Digital Content 1, http://links.lww.com/ALN/A856). Because the expected drug effect was suppression of guarding and the animals expressed only little of type 2 guarding, in the following sections the drug effects are reported only for type 1 guarding.

Although plantar incision induces heat as well as mechanical hypersensitivity, heat hypersensitivity was not assessed in the present study, because the TRPA1 channel is not involved in transduction of noxious heat. Therefore, cutaneous hypersensitivity was assessed only with mechanical stimulation. To assess mechanical hypersensitivity, the frequency of withdrawal responses to application of monofilaments (von Frey filaments) to the operated hind paw as well as to the contralateral (control) hind paw was examined. In the operated hind paw, the monofilament was applied to the skin area immediately adjacent to the sutured skin incision, whereas in the contralateral side the monofilament was applied to a corresponding area in the uninjured plantar skin. A series of calibrated monofilaments (North Coast Medical, Inc., Morgan Hill, CA) was applied in ascending order five times to the plantar skin at a frequency of 0.5 Hz. In the injured paw, monofilaments producing forces varying from 1–26 g were used, whereas in the uninjured paw monofilaments producing forces varying from 1–60 g were used. A visible lifting of the stimulated hind limb was considered a withdrawal response. If the rat failed to withdraw to any of the five presentations of a monofilament, the response rate for the studied force level was 0%. If the rat withdrew every time the monofilament was applied to the paw, the response rate for the studied force level was 100%. Thus, a decrease in the response rate represents suppression of mechanical stimulus-evoked pain behavior. Here we use the term mechanical hyperalgesia when the response rate is increased to mechanical stimulation at a force of 15 g or more, whereas the term tactile allodynia is used when referring to an increase in the response rate to mechanical stimulation at a force of 8 g or fewer. When referring to increased response rate to mechanical stimulation in general (independent of the stimulus force), the term mechanical hypersensitivity is used in this report. The term mechanical hypersensitivity overlaps with the terms mechanical hyperalgesia and tactile allodynia.

Skin incision induced a significant hypersensitivity to monofilament stimulation as shown by a significant increase in the withdrawal response frequency evoked by monofilament stimulation of the injured paw in a group of untreated animals (main effect of skin incision: F1,128 = 194, P < 0.0001; see panel B in Supplemental Digital Content 1, http://links.lww.com/ALN/A856, which is a graph showing the withdrawal response rates of the incised and the control hind limbs to mechanical stimulation before drug treatments). Mechanical hypersensitivity was reduced from PD1 to PD2 (main effect of postoperative day: F1,128 = 9.5, P = 0.0025; see panel C in Supplemental Digital Content 1, http://links.lww.com/ALN/A856, which is a graph showing the withdrawal response rate of the incised hind paw to mechanical stimulation on the first two postoperative days), but hypersensitivity in the incised paw was still highly significant on D2 when compared with the monofilament-induced response of a control limb (main effect of skin incision: F1,128 = 71.8, P < 0.0001; not shown).

**Preemptive Treatment**

To assess whether preemptive treatment with a TRPA1 channel antagonist attenuates development of postoperative pain and hyperalgesia, 30 μg saline or CHEM was administered intraplantarly to the hind paw 15 min before skin plus deep-tissue incision. After the operation, animals received no other treatments. Guarding and mechanical hypersensitivity were tested 24 h after the operation as described above. It was...
expected that if preoperative CHEM treatment of the incised skin area has a preemptive analgesic effect, then guarding and/or hypersensitivity in the CHEM-treated animals on PD1 is reduced when compared with the saline-treated group.

**Sedation/Locomotion**
To exclude the possibility that blocking the TRPA1 channel might suppress postoperative pain behavior because of sedative or motor effect, locomotion of intact animals treated with a high dose of CHEM was assessed in a modified open-field test. CHEM (30 mg/kg intraperitoneally) was administered 15 min before the assessment of locomotion started. For comparison, one group of animals was treated with vehicle and one with a sedative dose of a general anesthetic, pentobarbitone (20 mg/kg intraperitoneally; OrionPharma, Espoo, Finland). For assessment of locomotor activity, the animal was placed for 2 min in a three-compartment place-preference device (San Diego Instruments, San Diego, CA). During testing, the animal had free access to all three chambers of the device. Movement of the animal was recorded with a 4 × 16 photobeam array. The duration of spontaneous movement during the first 2 min in the device was used as an index of sedation/locomotion. Each animal was tested only once. It was expected that if blocking the TRPA1 channel induces sedation or motor impairment, then the CHEM-treated animals spend less time in moving around the three chambers of the device than vehicle-treated ones.

**Drug Administrations**
Intraplantar administrations of drugs control were performed at a volume of 30 μl using a 50-μl Hamilton syringe connected via polyethylene tubing to a 27-gauge hypodermic needle. With intraplantar administrations, the drug was administered into the wound area or the corresponding area in the contralateral control limb. For intrathecal administration, the drug control were microinjected with a 50-μl Hamilton microsyringe in a volume of 5 μl followed by a saline flush in a volume of 15 μl. In each experiment, the drug was dissolved in physiologic saline immediately before its intrathecal or intraplantar administration, or in 0.5% methylcellulose before its intraperitoneal administration. Because of solubility problems, the highest intrathecal dose of the TRPA1 channel antagonists was 10 μg. Physiologic saline at a corresponding volume was used for control injections.

**Course of the Study**
After the surgery, each animal participated in two experiments, one of which was performed 24 h (PD1) and the other 48 h after the operation (PD2). The order of testing different compounds was counterbalanced between the animals. In each treatment group, half of the animals were tested on PD1 and half on PD2. Guarding behavior and mechanical hypersensitivity were assessed once before the treatments (both on PD1 and PD2). Comparison of pretreatment behaviors on PD1 versus PD2 allowed excluding the possibilities that the baseline pain behaviors were different between the two postoperative test days or that the treatments on PD1 had long-term effects influencing pain behaviors still on PD2.

The drug doses and the time-points for testing the drug effects were chosen based on previous results. Our previous results indicate that intraperitoneal administration of CHEM produced a dose-related antihypersensitivity effect in diabetic animals at doses 10–30 mg/kg intraperitoneally and the effect was prominent 15–60 min after its administration. Previously, intrathecal administration of CHEM at the dose of 10 μg had proven effective in attenuating mechanical hypersensitivity of presumably central origin in various pain models, without influencing mechanical threshold of healthy controls, and the significant antihypersensitivity effect lasted, at least, from 15 to 60 min after intrathecal administration. Moreover, preliminary experiments of the present study had shown that intraplantar administration of CHEM at the dose of 30 μg is sufficient to reduce mechanical hypersensitivity, without influencing mechanical threshold of the healthy control limb, at least up to 30 min after its administration. Based on these observations, the doses of CHEM used in the present study were 10 and 30 mg/kg intraperitoneally, 10 and 30 μg intraplantarly, and 10 μg intrathecally. For comparison, A-967079 was administered at the doses of 10 and 30 mg/kg intraperitoneally, 30 μg intraplantarly, and 10 μg intrathecally. Guarding was assessed before and 20–30 min after drug injections. Mechanical hypersensitivity was assessed both in the injured and the uninjured contralateral paw before and 30 min after drug injections.

Results of intraperitoneal, intraplantar, and intrathecal saline-treated control groups were pooled, since the saline-induced changes in guarding or mechanical hypersensitivity were not significant among groups with intraperitoneal, intraplantar, and intrathecal administrations of saline (main effect of the route of vehicle administration on guarding: 2.2). To assess the effect of blinding, suppression of guarding and hypersensitivity induced by intraplantar administration of CHEM or saline was administered blinded in six animals and without blinding in six animals. Blinding (i.e., the experimenter was not aware whether the animal was treated with CHEM or saline) failed to influence the magnitude of drug effect on guarding (main effect of blinding on the drug-induced effect on guarding: 0.63; see panel E in Supplemental Digital Content 1, http://links.lww.com/ALN/A856, which is a graph showing the effect of blinding on the assessment of guarding behavior) or mechanical hypersensitivity (main effect of blinding on the drug-induced effect on mechanical hypersensitivity: 2.7; see panel D in Supplemental Digital Content 1, http://links.lww.com/ALN/A856, which is a figure showing the effect of blinding on the assessment of mechanical hypersensitivity). Therefore, results obtained in
experiments in which the experimenter was blinded were pooled with the results obtained in experiments in which she was not blinded. In the present study, ipsilateral intraplantar administrations of saline in six animals and CHEM at a dose of 30 μg in six animals were performed blinded (see panels E and D in Supplemental Digital Content 1, http://links.lww.com/ALN/A856).

When assessing drug-induced effects on guarding behavior, the drug effect on type 1 guarding was calculated in percent for each condition in the following way: 100 X (duration of guarding after drug treatment/duration of guarding before drug treatment). Guarding % values below 100% represent drug-induced suppressions in guarding. When assessing drug-induced effects on mechanical hypersensitivity, the drug effect on the cumulative response rate to a series of monofilaments was calculated in the following way: the cumulative response rate after drug treatment – the cumulative response rate before drug treatment. Drug-induced changes in cumulative response rates that were less than 0 represent drug-induced antihypersensitivity effects. In addition, drug-induced antihypersensitivity effects were assessed by comparing stimulus-response functions following intraplantar or intrathecal treatments with CHEM. When assessing preemptive effect induced by preoperative intraplantar administration of CHEM on guarding and hypersensitivity, however, the absolute duration of type 2 guarding and the absolute cumulative response rate to monofilament stimulation assessed 24 h after operation were compared between the saline and CHEM-treated groups.

Pharmacokinetics of CHEM
Pharmacokinetics of CHEM was studied in male Hannover-Wistar rats (n = 3). CHEM was administered at a dose of 30 mg/kg intraperitoneally in methylcellulose suspension. Concentrations in plasma and brain were measured 0.25, 0.5, 1, 2, 5, 8, 12, and 24 h after the drug administration. Briefly, (homogenized) samples were extracted with ethylacetate: hexane and evaporated to dryness. Samples were reconstituted to mobile phase (10 mM NH4-acetate pH 4.5; ACN 50:50). Isocratic chromatographic separations were conducted with Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA) equipped with Waters Sunfire C8, 2.1 × 150 mm, 3.5 μm column, and mass spectrometry detection with API 3000 MS/MS mass spectrometer (Applied Biosystems, Carlsbad, CA). Bioanalytical methods were validated according to fit-for-purpose requirements.

The free fractions of CHEM in rat plasma and brain homogenate were determined with rapid equilibrium dialysis apparatus (Pierce/Thermo Fisher, Rockford, IL). Briefly, rat plasma or brain homogenate (1 part brain + 4 parts phosphate buffer) spiked with CHEM were applied to plasma chamber and phosphate buffer to buffer chamber. Samples were equilibrated for 4 h, after which both chambers were analyzed for CHEM with liquid chromatography/mass spectrometry (peak area comparison, not quantitative analysis).

Free fractions were calculated and brain homogenate results were corrected for dilution factor as suggested by Kalvass et al. Because CHEM is neutral compound at physiologic pH range, the ion-trapping corrections suggested by Friden et al. were used.

Statistics
Data analysis was performed using one- or two-way ANOVA followed by Tukey post hoc test or Student t test with a Bonferroni correction for multiple comparisons (comparisons among three or more groups) or unpaired Student t test (comparisons between two groups). P < 0.05 (two-tailed) was considered to represent a significant difference. GraphPad Prism 4 software for Windows (GraphPad Software Inc., La Jolla, CA) was used for analyzing the data.

Results
Guarding Behavior
Guarding induced by skin plus deep-tissue incision was significantly reduced by intraperitoneal treatment with CHEM, a TRPA1 channel antagonist (main effect of intraperitoneally administered CHEM on guarding: F2,38 = 42.7, P < 0.0001; fig. 1A). Post hoc testing indicated that intraperitoneal treatment with CHEM reduced guarding at the dose of 30 mg/kg but not yet at the dose of 10 mg/kg. Also intraplantar treatment of the operated skin area with CHEM produced a significant reduction in guarding (main effect of intraplantarly administered CHEM on guarding: F2,38 = 27.8, P < 0.0001; fig. 1B). Post hoc tests indicated that guarding was significantly reduced following ipsilateral intraplantar treatment with CHEM at a dose of 30 μg but not at the dose of 10 μg (fig. 1B). To assess whether the suppression of guarding induced by ipsilateral intraplantar treatment with CHEM was because of a peripheral or systemic effect, we compared changes in guarding following intraplantar administration of 30 μg of CHEM into the hind paw ipsi- versus contralateral to the skin incision. Although there was a general effect by CHEM (main effect of intraplantarly administered CHEM on guarding in the ipsi- and contralateral limb: F2,38 = 30.9, P < 0.0001), post hoc tests indicated that intraplantar administration of CHEM (30 μg) suppressed guarding only when administered into the injured paw but not when it was administered into the contralateral paw (fig. 1C). Intrathecal administration of CHEM at the dose of 10 μg failed to suppress guarding (t27 = 1.9; fig. 1D).

A-967079, a TRPA1 channel antagonist with a structure different than CHEM, was used to verify that the CHEM-induced effects on guarding were because of block of the TRPA1 channel. Also intraperitoneal administration of A-967079 produced a dose-related suppression of guarding (main effect of intraperitoneally administered A-967079 on guarding: F2,32 = 5.08, P = 0.012; see panel A in Supplemental Digital Content 2, http://links.lww.com/ALN/A857, which...
is a graph showing guarding following intraperitoneal administration of A-967079). Post hoc testing indicated that the lowest intraperitoneal dose significantly suppressing guarding was 30 mg/kg (see panel A in Supplemental Digital Content 2, http://links.lww.com/ALN/A857). Moreover, ipsilateral intraplantar administration of A-967079 at the dose of 30 μg significantly suppressed guarding (t_{27} = 2.7, P = 0.0128; see panel B in Supplemental Digital Content 2, http://links.lww.com/ALN/A857, which is a graph showing guarding following intraplantar administration of A-967079), whereas intrathecal administration of A-967079 at the dose of 10 μg failed to produce a significant effect on guarding (t_{26} = 1.9; see panel C in Supplemental Digital Content 2, http://links.lww.com/ALN/A857, which is a graph showing guarding following intrathecal administration of A-967079).

**Mechanical Hypersensitivity**

Mechanical sensitivity was tested by assessing the frequency of the limb withdrawal response to repetitive application of a calibrated series of monofilaments immediately adjacent to the skin incision site or to a control site in the contralateral hind paw. Mechanical hypersensitivity was significantly attenuated by intraperitoneal treatment with CHEM (main effect of intraperitoneally administered CHEM on mechanical hypersensitivity: F_{2,32} = 21.1, P < 0.0001; fig. 2A). Post hoc testing indicated that intraperitoneal administration of CHEM at the dose of 30 mg/kg had a significant antihypersensitivity effect, whereas the dose of 10 mg/kg failed to suppress hypersensitivity (fig. 2A). Also intraplantar treatment of the injured paw produced a significant suppression of mechanical hypersensitivity (main effect of intraplantar treatment with CHEM on mechanical hypersensitivity: F_{2,38} = 24.4, P < 0.0001; fig. 2B). Post hoc tests indicated that the CHEM-induced antihypersensitivity effect was significant following intraplantar treatment of the injured paw at the doses of 10 and 30 μg (fig. 2B). The antihypersensitivity effect induced by ipsilateral intraplantar administration of a TRPA1 channel antagonist was because of a peripheral action, since intraplantar administration of CHEM at the dose of 30 μg produced a significant antihypersensitivity effect only when administered into the injured paw but not when it was administered into the contralateral hind paw (fig. 2C). Moreover, intrathecal administration of CHEM at the dose...
of 10 μg had a significant antihypersensitivity effect ($t_{27} = 5.9, P < 0.0001$; fig. 2D).

Intraperitoneal administration of A-967079 produced a significant mechanical antihypersensitivity effect in the operated paw (main effect of intraperitoneal administration of A-967079 on mechanical hypersensitivity: $F_{2,32} = 19.8, P < 0.0001$; see panel A in Supplemental Digital Content 3, http://links.lww.com/ALN/A858, which is a graph showing mechanical hypersensitivity following intraperitoneal administration of A-967079). Post hoc testing indicated that the lowest intraperitoneal dose of A-967079 producing a significant antihypersensitivity effect was 30 mg/kg. A-967079 produced a significant antihypersensitivity effect also when it was administered at the dose of 30 μg into the injured paw ($t_{27} = 3.6, P = 0.0012$; see panel B in Supplemental Digital Content 3, http://links.lww.com/ALN/A858, which is a graph showing mechanical hypersensitivity following intraplantar administration of A-967079) or at the dose of 10 μg intrathecally ($t_{27} = 4.4, P = 0.0002$; see panel C in Supplemental Digital Content 3, http://links.lww.com/ALN/A858, which is a graph showing mechanical hypersensitivity following intrathecal administration of A-967079).

To assess whether blocking the peripheral TRPA1 channel attenuates hypersensitive responses evoked by innocuous and noxious mechanical stimuli in a similar fashion as blocking the spinal TRPA1 channel, we assessed withdrawal responses evoked by monofilaments of different intensities following both ipsilateral intraplantar and intrathecal administrations of CHEM. Whereas the antihypersensitivity effect induced by CHEM was significant following both ipsilateral intraplantar (30 μg; main effect of intraplantarly administered CHEM: $F_{1,231} = 56.2, P < 0.0001$; fig. 3A) and intrathecal administrations (10 μg; main effect of intrathecally administered CHEM: $F_{1,189} = 25.4, P < 0.0001$; fig. 3B), it is noteworthy that ipsilateral intraplantar administration of CHEM predominantly suppressed responses evoked by noxious stimuli (10–26 g; fig. 3A). In contrast, the antihypersensitivity effect induced by intrathecal administration of CHEM was observed at test stimulus intensities that were lower than that at which blocking the peripheral TRPA1 channel produced its maximum effect (8 g or fewer; fig. 3B).

At the currently used doses, CHEM failed to influence mechanical sensitivity in the healthy control paw as shown by

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**Fig. 2.** Effect of a selective transient receptor potential ankyrin 1 channel antagonist (Chembridge-5861528) on mechanical hypersensitivity to monofilament stimulation in an experimental model of postoperative pain. (A) Intraperitoneal (i.p.) treatment. (B) Intraplantar (i.pl.) treatment ipsilateral to the injury. (C) Intraplantar treatment ipsi- versus contralateral to the injury. (D) Intrathecal (i.t.) treatment. In the Y-axis, 0% (shown by the dotted line) represents the cumulative withdrawal response rate to repetitive application of a series of monofilaments to the injured paw. Values less than 0% in the Y-axis represent a drug-induced antihypersensitivity effect. Graphs show medians, the boxes extend from the 25th to the 75th percentile, and the whiskers extend from the smallest to the largest value ($n = 6$, except $n_{sal} = 24$ and in graph B, $n = 12$). ***$P < 0.005$ (Tukey post hoc test; reference: the Sal group, unless specified). Sal = saline control.
the finding that intraperitoneal treatment with 30 mg/kg of CHEM failed to influence the withdrawal response evoked by mechanical stimulation of the uninjured control paw (t10 = 0.24; fig. 4A). Moreover, intraplantar administration of CHEM at a dose of 30 μg into the uninjured control paw failed to influence the withdrawal response evoked by its mechanical stimulation (t10 = 0.36; fig. 4B).

Preemptive Treatment

To assess whether preoperative block of the TRPA1 channels in the area of operation has a preemptive analgesic effect, the operated region in the hind paw was treated 15 min before the operation with CHEM or saline control. Assessment of pain behavior 24 h after the operation indicated that preoperative intraplantar administration of CHEM at the dose of 30 μg failed to reduce guarding (t10 = 1.0; fig. 5A) or mechanical hypersensitivity (t10 = 1.7; fig. 5B).

Sedation/Locomotion

To exclude the possibility that the CHEM-induced suppression of guarding or hypersensitivity were because of sedation or motor impairment, animals treated with 30 mg/kg of CHEM intraperitoneally were tested in the open-field test. For comparison, one group of animals was treated with a low dose of general anesthetic, pentobarbitone (20 mg/kg intraperitoneally), and one with vehicle. Drug treatments had a significant effect on duration of spontaneous locomotion in the open-field test (main effect of drug treatments: F2,15 = 7.3, P = 0.006; see figure in Supplemental Digital Content 4, http://links.lww.com/ALN/A859, which is a graph showing duration of locomotor activity in the open-field test). Post hoc testing, however, indicated that locomotion was decreased only in pentobarbitone-treated animals, whereas locomotor activity was of equal magnitude in CHEM-treated as vehicle-treated animals (see figure in Supplemental Digital Content 4, http://links.lww.com/ALN/A859).

Pharmacokinetics of Chembridge-5861528

Plasma concentrations of CHEM were close to 2,000 ng/ml from 0.25 to 2 h after its intraperitoneal administration at the dose of 30 mg/kg. Brain/plasma ratio was about 0.4. Fraction unbound at 1,000 ng/ml was 0.019 in plasma and 0.023 in brain homogenate (corrected for dilution). Therefore, free...
increment of a series of monofilaments to the injured area is shown; maximum value is 800%, and the higher the cumulative rate, the stronger the mechanical hypersensitivity. Graphs show medians, the 75th percentile, and the whiskers extend from the 25th to the 75th percentile, and the whiskers extend from the smallest to the largest value (n = 6). CHEM = Chembridge-5861528; i.pl. = intraplantar; Sal = saline.

plasma concentrations during first 2 h were about 40 ng/ml and free brain concentrations about 18 ng/g.

**Discussion**

A selective TRPA1 channel antagonist suppressed guarding and mechanical hypersensitivity induced by a skin plus deep-tissue incision. This finding indicates that the TRPA1 ion channel contributes both to maintenance of sustained postoperative pain and facilitation of mechanical stimulus-evoked pain. Importantly, blocking the cutaneous versus spinal TRPA1 channel differentially influenced guarding and mechanical hypersensitivity. Blocking the cutaneous TRPA1 channel in the injured paw reduced guarding and mechanical hyperalgesia, whereas blocking the spinal TRPA1 channel preferentially reduced tactile allodynia. The finding that intraplantar administration of the TRPA1 channel antagonist attenuated guarding and mechanical hyperalgesia only when administered into the injured paw, but not to a contralateral site, supports the interpretation that the suppression of pain behavior induced by ipsilateral intraplantar treatment was because of peripheral rather than systemic (or spinal) action. Moreover, ipsilateral intraplantar or systemic administration of the TRPA1 channel antagonist reduced mechanical hyperalgesia and guarding at a dose that failed to influence mechanical nociception in the uninjured control paw. It should, however, be noted that the TRPA1 channel does contribute to transduction of noxious mechanical stimuli in the healthy skin also, with a higher dose of the TRPA1 channel antagonist than that currently used producing mechanical antinociception in an uninjured site. 

**Potential Endogenous Agonists Driving the TRPA1 Channel in Postoperative Conditions**

The reduction in guarding and mechanical hyperalgesia following block of the peripheral TRPA1 channel supports the proposal that surgery induces release of endogenous TRPA1 channel agonists acting on the pronociceptive TRPA1 channel in the injured tissue. Previous studies indicate that lactate, low pH, and increased expression of the nerve growth factor are present in incisions. Because weak acids, such as lactate, activate the TRPA1 channel and the nerve growth factor increases expression of the TRPA1 channel on sensory neurons, these factors are among potential peripheral mechanisms for the TRPA1 channel-mediated facilitation of mechanically evoked responses and sustained pain.

In the spinal dorsal horn, plantar incision induces activation of microglia, which provides an important source of TRPA1 channel agonists, such as reactive oxygen species. Plantar incision-induced activation of microglia and the consequent increase of endogenous TRPA1 channel agonists in the spinal dorsal horn are expected to cause activation of the TRPA1 channel on central endings of nociceptive nerve fibers, which leads to facilitation of transmission. This may explain the spinal TRPA1 channel-mediated component of tactile allodynia.

A number of earlier studies have attempted to assess the role of the peripheral and the spinal TRPA1 channel in pain by local administrations of selective TRPA1 channel agonists. In one experimental human study, cutaneous application of cinnamaldehyde induced sustained pain and mechanical hyperalgesia, but no tactile allodynia in most of the subjects. Tactile allodynia has, however, accompanied pain and/or hyperalgesia induced by cutaneous application of cinnamaldehyde, allyl isothiocyanate, or 4-hydroxynonenal in other human and animal studies. Activation of spinal TRPA1 channels by intrathecal administration of cinnamaldehyde in a recent rat study induced mechanical hypersensitivity.
sensitivity that was most prominent at innocuous test stimulus intensities and that was not accompanied by marked signs of spontaneous pain, such as vocalizations.\textsuperscript{12} The pronociceptive effects induced by peripheral and spinal administrations of TRPA1 channel agonists are to a large extent in line with the present findings showing that the mechanical antihypersensitivity effect following block of the cutaneous TRPA1 channel was most prominent at high (noxious) stimulus intensities (mechanical antihyperalgesia), whereas blocking the spinal TRPA1 channel preferentially reduced responses elicited by low stimulus intensities (tactile antiallodynia). Our pharmacokinetic results indicate that at a high systemic dose CHEM may have produced its pain-suppressive actions because of blocking both the spinal and peripheral TRPA1 channels.

**TRPV1 versus TRPA1 Channel in Postoperative Pain**

There is abundant evidence indicating that the TRPV1 channel that is expressed on nociceptive nerve fibers has an important role in transduction of noxious stimuli.\textsuperscript{2–4} Although blocking of the TRPA1 channel in the present study reduced guarding and mechanical hypersensitivity, blocking the peripheral and central TRPV1 channel has reduced heat hypersensitivity but not guarding or mechanical hypersensitivity induced by plantar incision.\textsuperscript{36} On the other hand, pretreatment of the incision area with a low dose of capsaicin, a TRPV1 channel agonist, has reduced both postoperative heat hyperalgesia and guarding but not mechanical hypersensitivity.\textsuperscript{27} In contrast, administration of a high dose of capsaicin in the vicinity of the wound\textsuperscript{28} or perineural administration of resiniferotoxin,\textsuperscript{29} an ultrapotent TRPV1 agonist, has reduced development of mechanical as well as heat hypersensitivity. Results obtained with a selective TRPV1 channel antagonist are likely to reveal the functional role of the TRPV1 channel. Results obtained with a TRPV1 channel agonist, in contrast, may reflect functional role of the TRPV1 channel-expressing neuron. This because the TRPV1 channel agonist-induced effects may reflect desensitization or degeneration of TRPV1 channel-expressing nerve fibers, or central effects by TRPV1 channel agonist-induced pain.\textsuperscript{3–4} Together the earlier results with TRPV1 channel antagonists and agonists indicate that TRPV1 channel-expressing nerve fibers are critical for guarding pain and mechanical hypersensitivity but the TRPV1 channel is not, as proposed earlier.\textsuperscript{27} Because the TRPA1 channel is expressed on a subpopulation of TRPV1 channel-expressing nociceptive nerve fibers,\textsuperscript{1–4} this proposal is in line with findings of the present study.

**Peripheral and Central Mechanisms of Postoperative Pain and Hypersensitivity**

Previous studies indicate that skin incision-induced guarding, mechanical hyperalgesia, and tactile allodynia are based on at least partly different mechanisms.\textsuperscript{30–35} In the present study, blocking the peripheral TRPA1 channel in the injured paw attenuated guarding and mechanical hyperalgesia, which is in line with previous findings indicating that guarding and mechanical hyperalgesia are dependent on sustained inputs from the injured region.\textsuperscript{30,32,36} Moreover, the present finding that blocking the spinal TRPA1 channel attenuated tactile allodynia is in line with earlier findings suggesting that central mechanisms contribute to tactile allodynia or secondary mechanical hyperalgesia induced by skin incision.\textsuperscript{32,35} which is the case also in various other pathophysiological conditions.\textsuperscript{37} Cutaneous neurogenic inflammation is among conditions causing centrally mediated secondary hypersensitivity (or hyperalgesia) to mechanical stimulation. Interestingly, recent results suggest that cutaneous neurogenic inflammation induces secondary mechanical hypersensitivity that is dependent on the central (spinal) TRPA1 channel.\textsuperscript{10,12,38} In line with this, a study in humans indicated that secondary mechanical hypersensitivity is enhanced in subjects with a gain-of-function mutation in the gene coding the TRPA1 channel.\textsuperscript{39}

It should be noted that the failure to reduce guarding by intrathecal administration of the TRPA1 channel antagonist in the present study does not exclude contribution of spinal mechanisms to maintenance of sustained postoperative pain. Indeed, an earlier study showed that intrathecal as well as intraplantar administration of a low dose of ketoprofen selectively reduced plantar incision-induced guarding, indicating contribution of spinal as well as peripheral mechanisms.\textsuperscript{33} Nor do the present results exclude the possibility that at a dose higher than used, intrathecal administration of the TRPA1 channel antagonist had reduced guarding as well as mechanical hypersensitivity.

The mechanical antihypersensitivity effect by TRPA1 channel antagonists has been demonstrated in multiple pain models.\textsuperscript{2–4,38} In addition, previous studies have shown that administration of various exogenous compounds, such as formalin, allyl isothiocyanate, or cinnamaldehyde, produces sustained pain behavior that is mediated by the TRPA1 channel.\textsuperscript{30–42} However, it is noteworthy that, as far as we are aware of, this is the first study to demonstrate that blocking the TRPA1 channel attenuates ongoing (“spontaneous”) pain that is endogenously maintained.

**Conclusions**

The present results indicate that the peripheral TRPA1 channel is involved in postoperative guarding and mechanical hyperalgesia, whereas the spinal TRPA1 channel is involved in tactile allodynia. Both systemic and topical administrations of TRPA1 channel antagonists proved effective in reducing postoperative pain and hyperalgesia. Moreover, an antihyperalgesic dose of a TRPA1 channel failed to produce motor, sedative, or other obvious side-effects. Together, these findings indicate that blocking the TRPA1 channel might provide an effective therapy for postoperative pain conditions.
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References

suppression of postincisional pain by a slow-release formulation of lidocaine. Anesthesiology 2011; 114:135–49


42. Andrade EL, Luiz AP, Ferreira J, Calixto JB: Pronociceptive response elicited by TRPA1 receptor activation in mice. Neuroscience 2008; 152:511–20

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Patton’s 1903 Anaesthesia and Anaesthetics

Born in Ralston, Pennsylvania, and medically educated at the City University of New York, Joseph McIntyre Patton, M.D. (1860–1930), practiced in Chicago the rest of his life. As “Professor of Physical Diagnosis and General Anaesthesia” in the College of Dentistry at the University of Illinois, Patton published in 1903 the first (above) of at least three editions of his landmark text Anaesthesia and Anaesthetics, General and Local, for Practitioners and Students of Medicine and Dentistry. In the preface of his 204-page octavo, Patton stated his modest goal of providing a book “sufficiently concise to fit the opportunities of the average student or busy practitioner, yet complete enough to afford a fair and impartial resume of our present knowledge of the subject.” (Copyright © the American Society of Anesthesiologists, Inc.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA’s Wood Library-Museum of Anesthesiology, Park Ridge, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UJYC@aol.com.