

Effect of AMG0347, a Transient Receptor Potential Type V1 Receptor Antagonist, and Morphine on Pain Behavior after Plantar Incision

Chaoran Wu, M.D., Ph.D.,* Narendra R. Gavva, Ph.D.,† Timothy J. Brennan, M.D., Ph.D.‡

Background: Studies on postoperative pain examine the etiology of incisional pain with the goal to develop new treatments for patients' pain after surgery. The current study examined the analgesic effects of a recently developed transient receptor potential vanilloid 1 (TRPV1) antagonist, AMG0347, on incisional pain in rats. Doses of morphine lower than those used in most rodent studies were also examined.

Methods: Adult Sprague-Dawley rats were underwent plantar incision. The effect of either AMG0347 or morphine was tested for its effects on guarding pain score, heat withdrawal latency, and mechanical withdrawal threshold. AMG0347 was also tested against nociceptive behaviors caused by capsaicin.

Results: For incisional pain, AMG0347 did not change the withdrawal threshold to mechanical stimulation or the guarding pain score. The withdrawal latency to heat increased from 3 h through 1 day after AMG0347 administration. AMG0347 prevented the decreases in heat withdrawal latency and mechanical withdrawal threshold caused by capsaicin infiltration and prevented the increase in activity caused by intrathecal capsaicin injection. Doses of morphine less than 1 mg/kg inhibited both the guarding and heat hyperalgesia; only the 1-mg/kg does affected mechanical responses.

Conclusions: AMG0347 decreased capsaicin-induced heat and mechanical hyperalgesia and blocked central TRPV1 receptors. AMG0347 only decreased heat hyperalgesia after plantar incision even though both peripheral and central TRPV1 receptors were blocked. The smallest doses of morphine affected guarding pain and heat responses.

STUDIES on postoperative pain, postoperative pain mechanisms, and hyperalgesia caused by incisions have improved our understanding of pain after surgery and provided the rationale for future treatments. Our laboratory has studied mechanisms of incisional pain and sensitization of nociceptor pathways by surgical incisions. One goal of this research is to examine the etiology of incisional pain and develop new treatments for patients' pain after surgery. One putative target for future postoperative pain treatment is the capsaicin or transient receptor potential vanilloid 1 (TRPV1) receptor. TRPV1 is the first transient receptor potential to be cloned

among the TRP channels, which are expressed in both the peripheral and the central nervous system. In the peripheral nervous system, TRPV1 channels are found in small- to medium-diameter dorsal root ganglion neurons and on both central and peripheral terminals.^{1,2} In the central nervous system, TRPV1 channels are localized both presynaptically and postsynaptically in spinal cord,³ and in various regions of the brain.^{4,5} TRPV1 is a nonselective cation channel activated directly by noxious chemicals, such as capsaicin, noxious heat (> 43°C), and low pH (< 6.0), and also indirectly by a number of inflammatory factors, including nerve growth factor (NGF), bradykinin, lipids, and prostaglandins.⁶ Activation of TRPV1 results in an influx of cations, particularly Ca²⁺ and Na⁺ ions, depolarization, exocytosis of neuropeptides and excitatory amino acids,⁷ and induces nociception.^{8,9}

A previous study by Honore *et al.*¹⁰ demonstrated that a TRPV1 antagonist 1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)urea (A-425619) reversed exaggerated heat responses and inhibited mechanical hyperresponsiveness after plantar incision, suggesting that this class of drugs will be highly effective for pain after surgery. It has been proposed that the marked analgesic effect of this class of drugs is a result of antagonism of both peripheral and central TRPV1 receptors.¹¹

In this study, we evaluated the effect of a different TRPV1 antagonist, (E)-N-(7-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl)-3-(2-(piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)acrylamide (AMG0347), against a variety of pain behaviors after plantar incision. AMG0347 inhibits activation of the rat TRPV1 channel by heat (IC₅₀ = 0.2 nM), protons (IC₅₀ = 0.8 nM), or capsaicin (IC₅₀ = 0.7 nM) in a concentration-dependent manner. Intraperitoneal injection of 0.5 mg/kg produces robust hyperthermia in rats by blocking TRPV1 receptors in the viscera.¹² We also examined the effect of very low doses of parenteral morphine, the standard drug for treatment of postoperative pain in humans, against these same pain behaviors. Surprisingly, AMG0347 was rather ineffective in our nociceptive tests. Morphine, in quite low doses, was effective. We examined whether peripheral and central TRPV1 receptors were blocked by the doses of AMG0347 used in our experiments. Both peripheral and central TRPV1 receptors were blocked. Doses of morphine that are less than those typically used in rodent experiments were effective against pain behaviors, whereas the TRPV1 antagonist was not.

* Postdoctoral Scholar, Department of Anesthesia, ‡ Samir Gergis Professor and Vice Chair for Research, Departments of Anesthesia and Pharmacology, University of Iowa Hospitals and Clinics. † Senior Scientist, Department of Neuroscience Research and Discovery, Amgen, Thousand Oaks, California.

Received from the Department of Anesthesia, University of Iowa Hospitals and Clinics, Iowa City, Iowa. Submitted for publication October 21, 2007. Accepted for publication February 14, 2008. Supported by the Department of Anesthesia, University of Iowa, Iowa City, Iowa, and grant Nos. GM-55831 and GM-067752 (to Dr. Brennan) from the National Institutes of Health, Bethesda, Maryland. Dr. Brennan has served as a consultant to Amgen, Thousand Oaks, California.

Address correspondence to Dr. Wu: Department of Anesthesia, 3000 ML, University of Iowa Hospitals and Clinics, Iowa City, Iowa 52242. chaoran-wu@uiowa.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.

Materials and Methods

This study was approved by the institutional Animal Care and Use Committee at the University of Iowa, Iowa City, Iowa. Male Sprague-Dawley adult rats (Harlan, Indianapolis, IN) weighing 275–300 g were housed with a 12-h light-dark cycle. Food and water were available *ad libitum*. In all experiments, persons who performed the tests were blinded to the dose of drug or vehicle administered. All rats were killed at the end of the protocol with gradually increasing concentrations of carbon dioxide.

Experimental Procedures

Plantar Incision. The rat hind paw plantar incision model was performed as described.¹³ Briefly, a 1-cm longitudinal incision was made 0.5 cm from the end of heel through the skin and fascia of the plantar aspect of right hind paw during anesthesia of 1.5–2.0% isoflurane. The underlying flexor muscle was divided, and then the skin was sutured with 5.0 nylon. Antibiotic ointment was applied to the incision immediately after surgery. Sutures were removed at the end of postoperative day 2.

Intrathecal Catheterization. Intrathecal catheter placement was performed for subarachnoid drug administration.¹⁴ Briefly, the rat was placed in a kyphotic position. A 3-cm longitudinal lumbar skin incision was made along the midline at the level of the iliac crests. The lumbar fifth and sixth intervertebral space was punctured with a 23-gauge hypodermic needle. Penetration into the subarachnoid space was indicated by a tail flick or hind paw retraction. A 10-cm-long 32-gauge polyurethane catheter (Micor, Allison Park, PA) was threaded through the needle cranially for approximately 3 cm. The 32-gauge catheter was fixed to the fascia and connected to a PE-10 catheter (Becton Dickinson, Sparks, MD) and tunneled under the skin to the nape of the neck. The catheter was flushed with saline and closed by heating. The dead space of the catheter was approximately 5 μ l. A lidocaine test (2% lidocaine, 20 μ l, intrathecally) was performed 30 min after catheterization to confirm the intrathecal catheter location. Experiment began on day 3 after intrathecal catheterization. At the end of the experiment, 30 μ l methylene blue dye was injected intrathecally followed by spinal dissection to confirm the location of the intrathecal catheter. Two rats were excluded because dye was not present in the spine after dye injection.

Subcutaneous Capsaicin Injection. Capsaicin (Sigma, Saint Louis, MO) was dissolved in 1.5% Tween 80, 1.5% alcohol, and saline. Capsaicin injection was performed on the plantar hind paw in rats that were briefly anesthetized with 2% isoflurane. A 30-gauge needle was inserted subcutaneously at the midline of right hind paw and directed toward the heel. The penetration site was 1.5 cm distal to the end of the heel. Capsaicin

(0.1%) or vehicle was injected subcutaneously in a total volume of 10 μ l.

Intrathecal Capsaicin Injection. Capsaicin was diluted to 0.01% with the same solvent as described in the Subcutaneous Capsaicin Injection section. Rats were placed into the activity chamber before injection. A total volume of 15 μ l capsaicin, 0.01%, was injected intrathecally using a Hamilton syringe (Hamilton Company, Reno, NV) to guarantee that 10 μ l capsaicin was injected into the subarachnoid space. The syringe was released from the catheter immediately after injection, and a locomotor test (described in the Locomotor Activity section) was started. In preliminary studies, capsaicin caused increased activity for 30 s. We recorded activity for 5 min and reported the activity for 1 min in 30-s bins.

Pain Behaviors

Guarding Behaviors. A cumulative pain score was used to assess nonevoked pain behaviors as described previously.¹⁵ Unrestrained rats were placed on a plastic mesh floor (8 \times 8 mm). The incised and nonincised paws were viewed. Both paws of each animal were closely observed during a 1-min period repeated every 5 min for 1 h. Depending on the position in which each paw was found during the majority of the 1-min scoring period, a 0, 1, or 2 was given. Full weight bearing of the paw (score = 0) was present if the wound was blanched or distorted by the mesh. If the paw was completely off the mesh without any touch, a score of 2 was recorded. If the area of the wound touched the mesh gently without any blanching or distorting, a 1 was given. The sum of the 12 scores (0–24) obtained during the 1-h session for each paw was obtained. The difference between the scores from the incised paw and nonincised paw was the cumulative pain score for that 1-h period.

Responses to Heat. Withdrawal latencies to heat stimuli were assessed by applying a focused radiant heat source on an unrestrained rat placed on a heat-tempered glass floor. The heat stimulus was a light from a 50-W projector lamp, with an aperture diameter of 6 mm, applied from underneath the glass floor (3 mm thick) on the middle of the incision. The latency time to evoke a withdrawal response was determined with a cutoff value of 30 s. Three trials 5–10 min apart were used to obtain average paw withdrawal latency.

Responses to Mechanical Stimuli. Rats were placed on an elevated plastic floor covered with a clear plastic cage top. The animals were allowed to ambulate, explore, and eventually rest lying on the mesh. For testing pain behaviors, von Frey filaments (thin plastic filaments with calibrated forces) were applied underneath the cage adjacent to the wound. Each filament was applied once starting with 10 mN and continuing until a withdrawal response occurred or 250 mN was reached. If a rat did not respond to the 250-mN filaments, 522 mN was recorded as the next filament. This was repeated a

total of three times with a 10-min test-free period between withdrawal responses. The lowest force from the three tests producing a response was considered the withdrawal threshold.

Locomotor Activity. Rats were placed individually in transparent plastic boxes ($17 \times 5 \text{ in}^2$) that were inserted into a SmartFrame Cage Rack MotorMonitor System (Kinderscientific, Poway, CA). This system can record the number of movements during the specified period with a dedicated microprocessor and infrared photo-beam technology. As the rat moves, it breaks photo-beams on an X-Y grid. There are 5 beams on the X grid and 17 beams on the Y grid. These beam breaks are recorded by the dedicated microprocessor. The collected data are then transmitted to a host computer. Rat activity was counted by recording the total number of movements in 30-s bins.

Experimental Protocols

Effect of AMG0347 on Pain Behaviors after Plantar Incision. For pain behaviors, rats were acclimated to the testing area for 2 h each day for 2 days to habituate to the testing environment.

Rats were randomly divided into four groups based on AMG0347 doses (vehicle, 1 mg/kg, 3 mg/kg, and 10 mg/kg). The first baselines were recorded before incision. For the von Frey filament test and heat test, the second baselines were recorded 1 h after incision. Then AMG0347 was administered orally immediately after the second baseline recordings. Mechanical and heat responses were measured 3 and 5 h after drug administration and daily for 2 days.

The effect of AMG0347 on the guarding pain was tested on postoperative day 1 in a separate group of rats. The second baseline was recorded the morning after incision followed by oral administration of AMG0347 (vehicle, 1 mg/kg, 3 mg/kg, and 10 mg/kg). Guarding behavior was recorded at 3 h, 5 h, 1 day, and 2 days after AMG0347 administration.

In a separate group of rats, we sought to determine whether we could pretreat rats to prevent the development of guarding pain behavior. Based on the onset and duration of drug effect against heat hyperalgesia, we administered AMG0347 (vehicle, 1 mg/kg, 3 mg/kg, and 10 mg/kg) 2 h before incision to 24 rats (6 rats per group). Guarding pain scores were measured 1 and 3 h after drug administration, at 8 AM and 1 PM the next day, and daily for 2 days.

Effect of AMG0347 on Heat Withdrawal Latency and Mechanical Withdrawal Threshold after Hind Paw Capsaicin Infiltration. The effect of AMG0347 on heat withdrawal latency was examined in 16 normal rats that received AMG0347 (vehicle, 1 mg/kg, 3 mg/kg, or 10 mg/kg). Baseline heat withdrawal latency was measured. Rats were given AMG0347 orally. Withdrawal

latency was recorded at 3 h, 5 h, and 1 day after drug administration.

Twelve rats were used to determine whether a minimal dose of capsaicin (0.1% for 10 μl) influences the heat withdrawal latency 30 min after intraplantar injection. Baseline heat withdrawal latency was measured. Capsaicin was injected, and withdrawal latency was recorded 30 min after capsaicin injection.

Twenty rats were used to examine the effect of AMG0347 on heat responses in rats after intraplantar capsaicin injection. Baseline heat withdrawal latency was measured. Then, AMG0347 (vehicle, 1 mg/kg, 3 mg/kg, or 10 mg/kg) was administered orally. Capsaicin (0.1% for 10 μl) was injected in the hind paw 2.5 h after AMG0347 administration. Withdrawal latency was recorded 30 min after capsaicin injection.

Twelve rats were used to examine the mechanical withdrawal threshold after intraplantar injection of capsaicin. Baseline withdrawal threshold was measured. Then, capsaicin (0.1% for 10 μl) was injected subcutaneously. Withdrawal threshold was measured 60 min later. Another 18 rats were used to examine the effect of AMG0347 on mechanical responses after intraplantar capsaicin injection. Baseline withdrawal threshold was measured. Then, AMG0347 (vehicle, 1 mg/kg, or 10 mg/kg) was administered orally. Capsaicin (0.1% for 10 μl) was injected 2 h after AMG0347 administration. Withdrawal threshold was recorded 60 min after capsaicin injection.

Effect of AMG0347 on Locomotor Activity after Intrathecal Capsaicin Injection. Twenty-two rats were used to examine the effect of AMG0347 on activity induced by intrathecal capsaicin (0.01% for 10 μl) administration. Rats were habituated to the recordings chambers for 2 days by placing them in the chamber for 5 min. Baseline activity was recorded 1 day before injection. The next day, AMG0347 (vehicle, 1 mg/kg, 3 mg/kg, or 10 mg/kg) was administered orally. Intrathecal capsaicin administration was performed 3 h after AMG0347 administration. Locomotor activity was recorded immediately after intrathecal capsaicin for 1 min.

Effect of Morphine on Incisional Pain Behaviors. Because of a relatively short duration of action of morphine, only one behavior was tested on a given day. Incisions were made, and rats recovered from surgery. On postoperative day 1, morphine (vehicle, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, and 1.0 mg/kg) was administered subcutaneously. Thirty minutes later, guarding pain was measured for the next hour. The next day, in the same rats, morphine (vehicle, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, and 1.0 mg/kg) was administered subcutaneously. Thirty minutes later, heat withdrawal latency was measured. Rats received the same dose of drug on postoperative days 1 and 2. We chose this order of testing because the magnitude of guarding pain is great enough

to test against drug treatment on postoperative day 1, but heat is robust for several days.¹³

Similarly, a separate group of rats underwent plantar incision. On postoperative day 1, morphine (vehicle, 0.1 mg/kg, 0.3 mg/kg, and 1.0 mg/kg) was administered subcutaneously. Thirty minutes later, mechanical withdrawal threshold was measured. On postoperative day 2 in the same group of rats, morphine (vehicle, 0.1 mg/kg, 0.3 mg/kg, and 1.0 mg/kg) was administered subcutaneously. Thirty minutes later, mechanical withdrawal threshold was measured. Rats received the same drug on postoperative days 1 and 2. Withdrawal responses to mechanical stimuli are robust on postoperative days 1 and 2.

Drug Administration. AMG0347 was a gift from Amgen Inc. (Thousand Oaks, CA). AMG0347 was dissolved in Oral Suspending Vehicle containing carboxymethylcellulose (Paddock Labs, Minneapolis, MN) and administered orally. Morphine was purchased from Baxter Healthcare Corp (Deerfield, IL). Capsaicin was purchased from Sigma (Sigma, St. Louis, MO).

Statistical Analyses

All statistical analyses were performed using Prism 4.0 (GraphPad Software, Inc., San Diego, CA). Withdrawal threshold to mechanical stimuli were expressed as median and interquartile range. Differences were determined by Friedman analysis of variance and Kruskal-Wallis test followed by the Dunn test for comparing paw withdrawal threshold after drug to vehicle. Mann-Whitney test was used for two independent group comparisons.

For guarding pain scores, withdrawal latencies to heat, and locomotor activity, data were expressed as mean \pm SEM. Two-way analysis of variance and one-way analysis of variance followed by the Dunnett test were used for comparisons *versus* vehicle. An unpaired *t* test was performed for two group comparisons. $P < 0.05$ was considered statistically significant.

Results

Effects of AMG0347 on Pain Behaviors Caused by Plantar Incision

The median withdrawal threshold to von Frey filament application was 12.8 mN 1 h after incision in all rats (fig. 1A). AMG0347 dosages of 1, 3, and 10 mg/kg did not change the withdrawal threshold to mechanical stimulation compared with vehicle-treated rats.

The mean withdrawal latency to radiant heat was 4.7 ± 0.3 s at 1 h after incision in all rats (fig. 1B). After vehicle treatment, the withdrawal latency was 6.3 ± 0.7 s at 3 h, 6.8 ± 0.6 s at 5 h, 7.8 ± 0.7 s at 1 day, and 16.5 ± 1.4 s at 2 days. All three doses of AMG0347 increased the heat withdrawal latency significantly compared with the vehicle group at 3 h ($P < 0.01$), 5 h ($P <$

0.01), and 1 day ($P < 0.05$) after drug. The maximum increase was 13.2 ± 1.0 s at 5 h after 3 mg/kg.

For guarding pain behavior, the mean cumulative pain score increased from 0.1 ± 0.2 to 12.4 ± 1.3 at 1 day after incision in all rats (fig. 1C). There was a significant group effect; however, drug only decreased the guarding pain behavior compared with vehicle at 1 day after 3 mg/kg ($P < 0.05$). After pretreatment of AMG0347 2 h before incision, there was no significant reduction in guarding pain behavior compared with the vehicle-treated group at 1 h, 3 h, 1 day, and 2 days after incision (fig. 1D).

Effect of AMG0347 on Heat and Mechanical Responses after Hind Paw Capsaicin Infiltration

The withdrawal latency to radiant heat was 12.0 ± 0.7 s in unincised rats before AMG0347 administration. All doses of AMG0347 (1 mg/kg, 3 mg/kg, and 10 mg/kg) did not change the withdrawal latency at any time after drug administration (fig. 2A).

Thirty minutes after hind paw capsaicin injection, the heat withdrawal latency decreased from 10.8 ± 0.8 s to 7.1 ± 0.5 s ($P < 0.01$; fig. 2B). AMG0347 significantly affected the capsaicin response. Doses of 1 mg/kg and 3 mg/kg AMG0347 prevented the decrease in the withdrawal latency caused by capsaicin injection ($P < 0.05$ *vs.* vehicle; fig. 2C).

The median withdrawal threshold to von Frey filament application was 586 mN before capsaicin injection. Both vehicle injection and capsaicin decreased the withdrawal threshold to 113.9 mN and 57.0 mN 60 min after hind paw injection, respectively. The withdrawal threshold after capsaicin was less than vehicle ($P < 0.01$; fig. 3A). One and 10 mg/kg AMG0347 increased the median withdrawal threshold to 114.4 and 141.7 mN, respectively. This was greater than the 70.1-mN withdrawal threshold after capsaicin in vehicle-treated rats ($P < 0.05$; fig. 3B). The remaining mechanical hyperalgesia after injection seems to be related to the needle injury and/or vehicle.

Effect of AMG0347 on Activity after Intrathecal Capsaicin Administration

The number of baseline movements in rats acclimated to the activity cages was 52.2 ± 7.0 during the first 30 s for all rats 1 day before the intrathecal capsaicin test. The next day, after oral vehicle pretreatment followed by intrathecal vehicle injection, the number of movements was 45.7 ± 10.0 30 s later. After oral vehicle treatment, intrathecal capsaicin increased the number of movements to 101.0 ± 23.0 during the first 30 s ($P < 0.01$). AMG0347 at 1 mg/kg and 10 mg/kg decreased the number of movements to 60.6 ± 9.2 and 37.8 ± 6.7 during the first 30 s after intrathecal capsaicin (fig. 4A). In the oral vehicle-treated group, activity from 30

to 60 s after capsaicin had returned to 35.8 ± 10.4 , the same as control rats (30.3 ± 5.7). AMG0347 treatment did not affect the activity after the intrathecal capsaicin response had abated (fig. 4B). The activity was 47.0 ± 12.8 and 27.3 ± 9.2 for the 1-mg/kg AMG0347 group and 10-mg/kg AMG0347 group, respectively.

Effect of Morphine on Incisional Pain Behaviors

The mean cumulative pain score for guarding behavior was -0.8 ± 0.3 ($n = 8$) before plantar incision. One day after incision, the cumulative pain score increased to 12.2 ± 1.1 ($n = 10$) 30 min after saline vehicle injection. The cumulative pain score decreased (fig. 5A) to 7.0 ± 2.3 ($n = 7$), 6.6 ± 1.1 ($n = 8$), 5.6 ± 0.6 ($n = 8$), and 3.0 ± 0.7 ($n = 8$) 30 min after 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, and 1.0 mg/kg morphine administration. All doses were different from vehicle.

The mean withdrawal latency to radiant heat was 12.9 ± 0.3 s ($n = 8$) before incision and decreased to 3.9 ± 0.2 s

($n = 10$) 30 min after saline injection on postoperative day 2. The withdrawal latencies were 5.6 ± 0.3 , 5.6 ± 0.7 , 7.2 ± 0.8 , and 8.2 ± 0.6 s at 30 min after 0.03 mg/kg ($n = 7$), 0.1 mg/kg ($n = 8$), 0.3 mg/kg ($n = 8$), and 1.0 mg/kg ($n = 8$) morphine injection (fig. 5B), respectively. Morphine (0.3 mg/kg and 1.0 mg/kg) increased the withdrawal latency significantly compared with saline.

The median withdrawal threshold to von Frey filament application was 17.7 mN 30 min after saline injection ($n = 8$) on postoperative day 1 (fig. 5C). After 0.1 mg/kg ($n = 8$) and 0.3 mg/kg ($n = 8$) morphine, the withdrawal thresholds were 30.8 and 46.2 mN, respectively, and were not different from control. The withdrawal threshold increased significantly to 78.8 mN 30 min after 1.0 mg/kg morphine ($n = 8$) administration compared with saline ($P < 0.0001$ vs. vehicle). Similar results were obtained on postincision day 2 (fig. 5D).

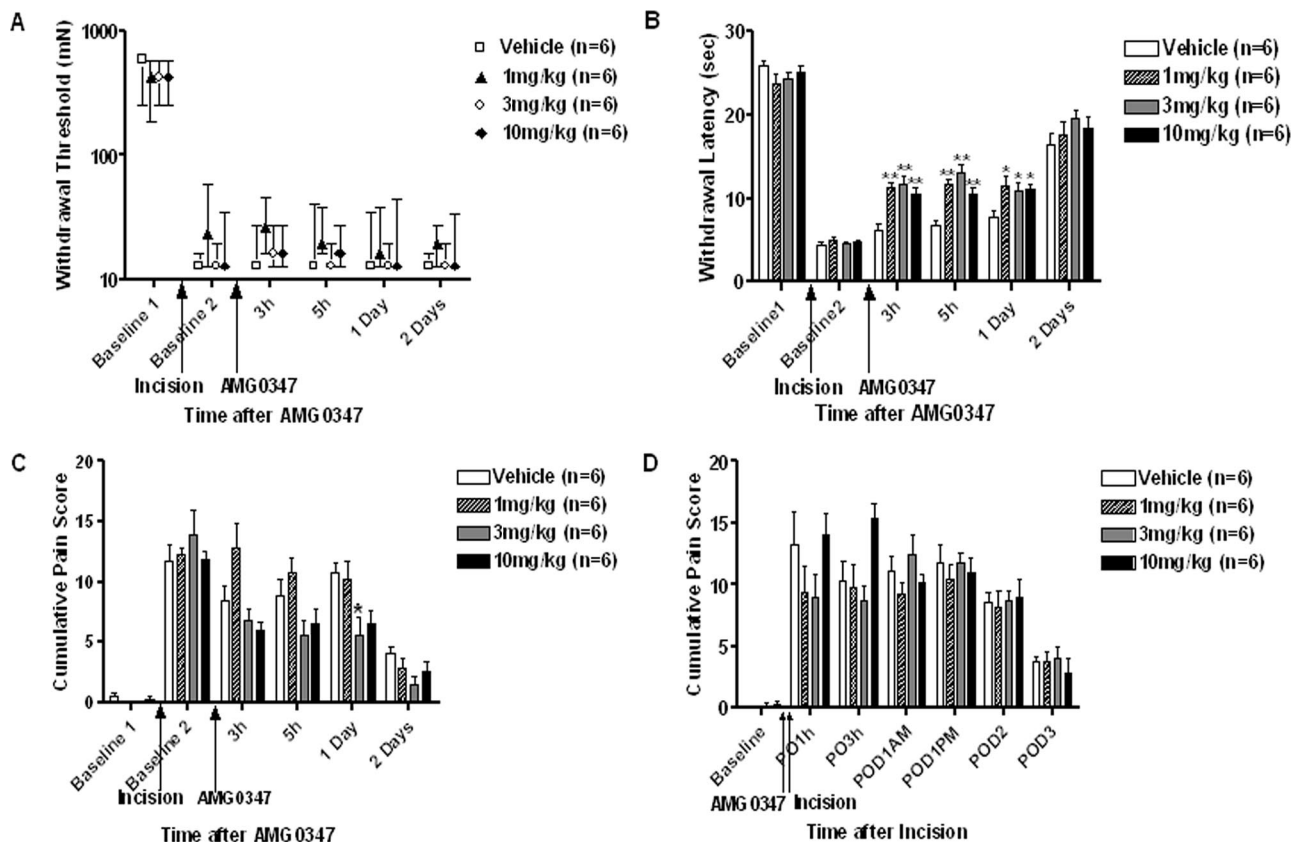


Fig. 1. Effect of AMG0347 on pain behaviors caused by plantar incision. (A) Withdrawal threshold to von Frey filament application. Fr (Friedman test score) = 5.2, $P = 0.16$, Friedman analysis of variance (ANOVA). Data are expressed as median and interquartile range (25th–75th percentile). (B) Withdrawal latency to radiant heat ($F_{15,100} = 3.0$, $P = 0.0006$, two-way ANOVA). At 3 h ($F_{3,20} = 9.5$, $P = 0.0004$, one-way ANOVA), 5 h ($F_{3,20} = 14.3$, $P < 0.0001$, one-way ANOVA), and 1 day ($F_{3,20} = 4.0$, $P = 0.02$, one-way ANOVA), there was drug effect versus vehicle. Data are expressed as mean \pm SEM. * $P < 0.05$ versus vehicle. ** $P < 0.01$ versus vehicle. (C) Guarding behavior and drug administration after incision ($F_{15,100} = 2.8$, $P = 0.0011$, two-way ANOVA). At 3 h ($F_{3,20} = 5.5$, $P = 0.006$, one-way ANOVA, Dunnett = not significant vs. vehicle), 5 h ($F_{3,20} = 3.5$, $P = 0.03$, one-way ANOVA, Dunnett = not significant vs. vehicle), 1 day ($F_{3,20} = 4.0$, $P = 0.02$, one-way ANOVA, Dunnett = significant vs. 3 mg/kg AMG0347), and 2 days ($F_{3,20} = 1.7$, $P = 0.19$, one-way ANOVA) after AMG0347 treatment. * $P < 0.05$ versus vehicle. (D) Drug administration before incision ($F_{18,120} = 2.1$, $P = 0.0104$, two-way ANOVA). At 1 h ($F_{3,20} = 1.4$, $P = 0.26$, one-way ANOVA), 3 h ($F_{3,20} = 3.8$, $P = 0.03$, one-way ANOVA, Dunnett = not significant vs. vehicle), 1 day (AM: $F_{3,20} = 1.5$, $P = 0.25$, one-way ANOVA; PM: $F_{3,20} = 0.3$, $P = 0.79$, one-way ANOVA), and 2 days ($F_{3,20} = 0.1$, $P = 0.96$, one-way ANOVA) after incision. Data are expressed as mean \pm SEM. AMG0347, TRPV1 antagonist. PO = postoperative; POD = postoperative day.

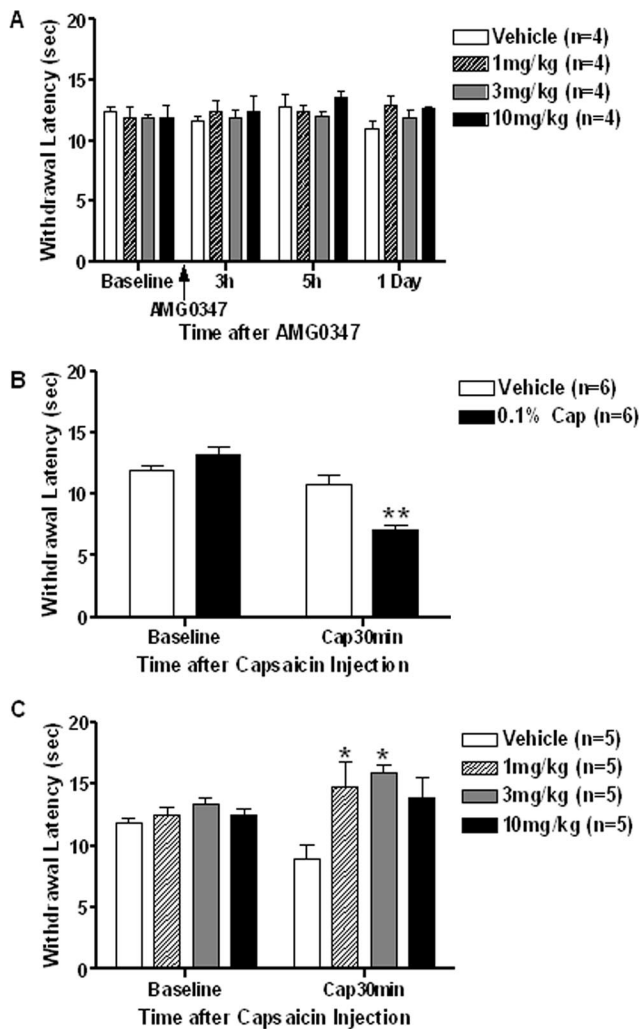


Fig. 2. Effect of AMG0347 on heat responses in normal rats and in rats after intraplantar capsaicin (Cap) injection. (A and B) Withdrawal latency to radiant heat in normal rats after AMG0347 (A; $F_{9,36} = 0.6$, $P = 0.77$, two-way analysis of variance) and in rats after intraplantar capsaicin or vehicle (B; $F_{1,10} = 40.3$, $P < 0.0001$, two-way analysis of variance; $t(10) = 4.2$, $P = 0.0018$). ** $P < 0.01$ versus vehicle. (C) Effect of AMG0347 on heat withdrawal latency after intraplantar capsaicin ($F_{3,16} = 3.3$, $P = 0.0469$, two-way analysis of variance; $F_{3,16} = 4.4$, $P = 0.02$, one-way analysis of variance, Dunnett vs. vehicle). Data are expressed as mean \pm SEM. * $P < 0.05$ versus vehicle.

Discussion

AMG0347 decreased postoperative heat hyperalgesia from 3 h through 1 day after drug administration. AMG0347 did not reduce the guarding pain behavior and mechanical hyperalgesia induced by hind paw incision. The doses of AMG0347 used block both central and peripheral TRPV1 receptors. In contrast, morphine affected postoperative guarding, heat, and mechanical hyperalgesia, although only the highest dose of morphine diminished the mechanical responses. These findings demonstrate that AMG0347 has limited analgesic efficacy compared with low doses of morphine after plantar incision and suggests that even though both peripheral and central TRPV1 receptors were blocked, antagonism

of TRPV1 receptors may not be an ideal analgesic strategy for postoperative pain treatment. The low doses of morphine show efficacy against incisional pain behaviors in smaller amounts than most studies of morphine in preclinical models.

There are many factors that can contribute to activation of the TRPV1 receptor. Some of these include NGF, low pH, heat, prostaglandins, and bradykinin.¹⁶ Some of these factors are also present after plantar incision and could contribute to postoperative pain *via* activation of TRPV1. NGF is increased in incised tissue and contributes to guarding pain and heat hyperalgesia after plantar incision.¹⁷⁻¹⁹ NGF may bind to its receptor, TrkA, and increase TRPV1 activity *via* several pathways, including phosphorylation of TRPV1 and protein kinase A, and phosphatidylinositol 3 or phospholipase C- γ signaling pathways.²⁰⁻²³ Although blocking NGF affects both guarding pain and heat hyperalgesia, results from the current study indicate that the inhibition of guarding behavior is not through an action of NGF at the TRPV1 receptor; other receptor systems must be involved. It is possible that the NGF in incisions activates the TRPV1 receptor to contribute to heat hyperalgesia. The extent of inhibition of heat hyperalgesia by TRPV1 receptor blockade and NGF sequestration are similar.

Decreased pH occurs after plantar incision, but the role of acid in incision pain is not yet understood. Injection of low-pH solutions into human skin produces pain and mechanical hyperalgesia.²⁴ A decrease in pH occurs after rat plantar incision and correlates to postoperative guarding pain, heat, and mechanical hyperalgesia, but again, demonstration of a role is not yet established.²⁵ Although protons may contribute to pain *via* activation of TRPV1,²⁶ other receptors activated by low pH may be involved as well.

Generally, TRPV1 does not respond to body temperature, but it is typically activated by heat in the range of 43°–52°C. In incisions, as opposed to inflammation, skin temperature is not increased. Therefore, theoretically, temperature is another local factor that may contribute to postoperative pain *via* TRPV1. Other factors, such as NGF and protons, may decrease the heat threshold of the TRPV1 to less than 43°C so that body temperature can activate the TRPV1 receptor.²⁷ Therefore, the TRPV1 receptor in incisions in the presence of NGF and acid could respond to lower temperatures, like 35°–42°C, and produce heat hyperalgesia.²⁵ In addition, if factors present in incisions decreased the heat threshold to below body temperature,²⁸ body temperature activating TRPV1 could contribute to spontaneous activity of nociceptors and unprovoked pain after incision. However, because guarding pain was not affected by TRPV1 receptor blockade, the TRPV1 receptor does not contribute to guarding, nonevoked pain behavior even though NGF sequestration does affect this.¹⁸

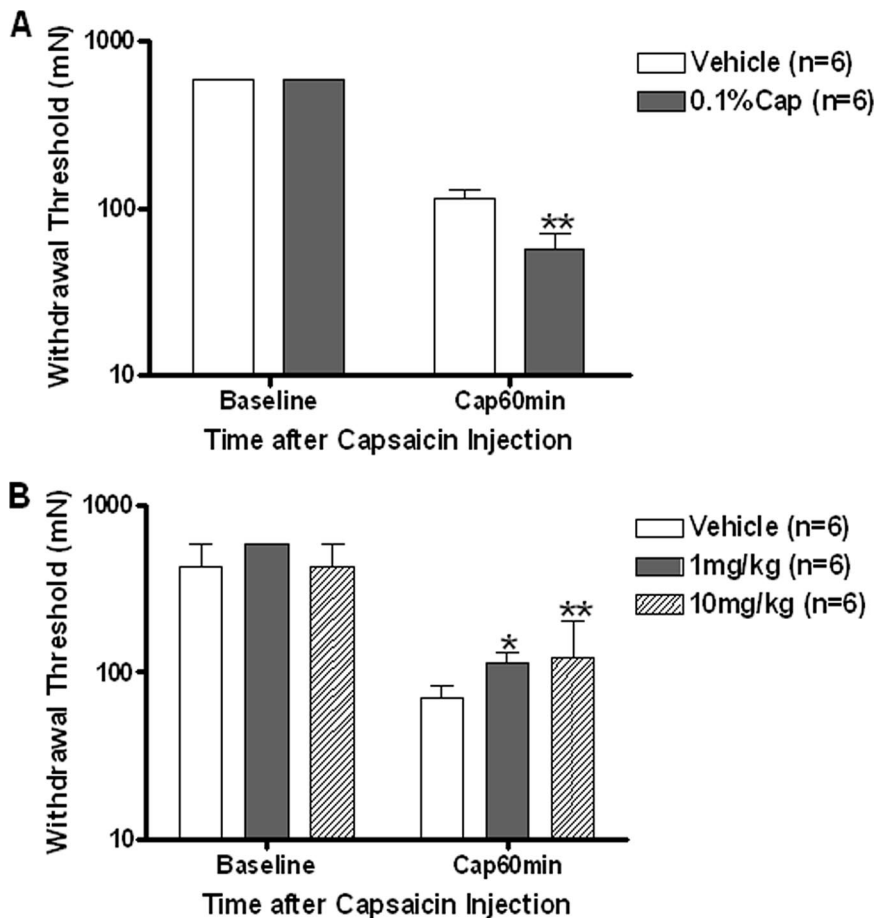


Fig. 3. Effect of AMG0347 on mechanical responses in rats after intraplantar capsaicin (Cap) injection. (A) Withdrawal threshold to von Frey filament application in rats after intraplantar capsaicin or vehicle ($MW_{10} = 0$, $P = 0.0022$, Mann-Whitney test). ** $P < 0.01$ versus vehicle. (B) Effect of AMG0347 on withdrawal threshold after intraplantar capsaicin ($KW_{2,15} = 10.27$, $P = 0.0059$, Dunn vs. vehicle). Data are expressed as median and interquartile range (25th–75th percentile). * $P < 0.05$ versus vehicle. ** $P < 0.01$ versus vehicle.

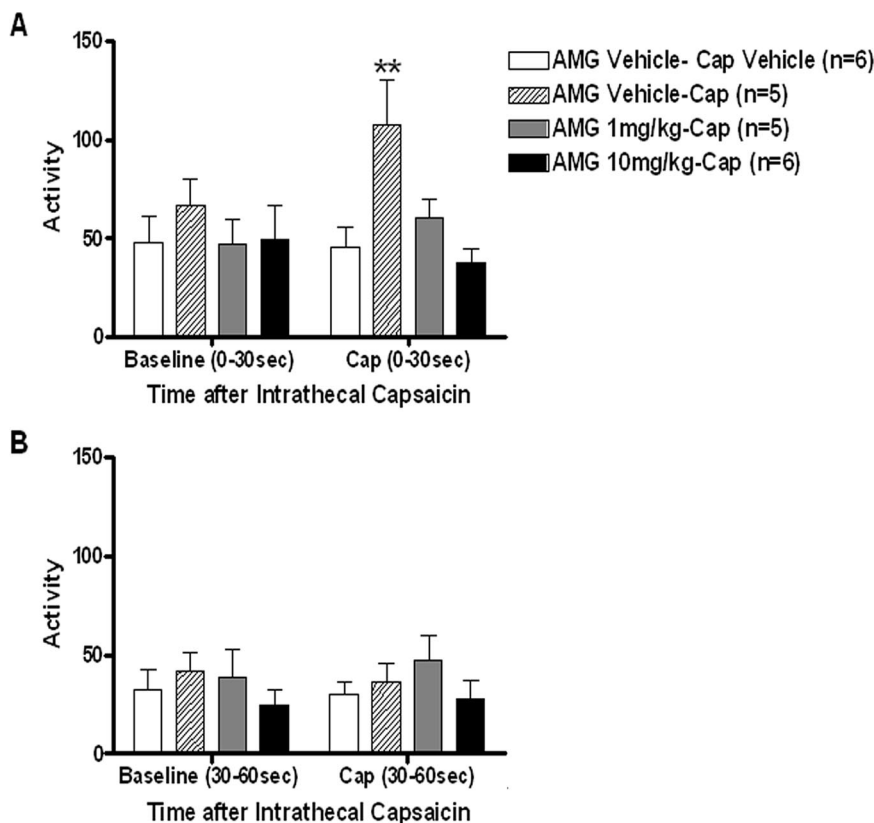
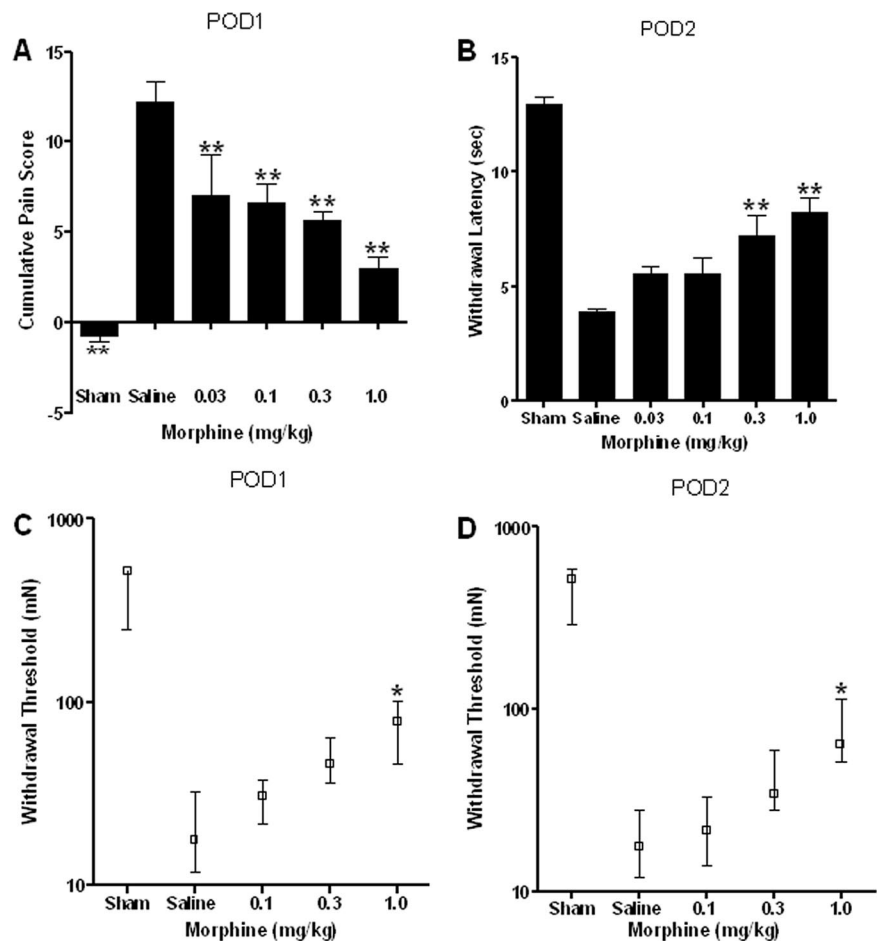


Fig. 4. Effect of AMG0347 on activity after intrathecal capsaicin (Cap) administration. (A) Baseline activity during the first 30 s on the day before and during intrathecal capsaicin injection showed only effect of drug ($F_{3,18} = 3.5$, $P = 0.0375$, two-way analysis of variance). Activity before ($F_{3,18} = 0.4$, $P = 0.76$, one-way analysis of variance) and activity during the first 30 s after intrathecal capsaicin ($F_{3,18} = 5.5$, $P = 0.007$, *post hoc* Dunnett vs. vehicle). ** $P < 0.01$ versus intrathecal vehicle group. (B) Activity baseline from 30 to 60 s on the day before intrathecal capsaicin injection. Activity 30 to 60 s after intrathecal capsaicin ($F_{3,18} = 0.2$, $P = 0.8773$, two-way analysis of variance). All data are expressed as mean \pm SEM.

Fig. 5. Effect of morphine on pain behaviors caused by plantar incision. (A) Guarding behavior 1 day after incision ($F_{5,43} = 15.9$, $P < 0.0001$, *post hoc* Dunnett). Data are expressed as mean \pm SEM. ** $P < 0.01$ versus saline group. (B) Withdrawal latency to radiant heat on postoperative day (POD) 2 in the same group shown in A ($F_{5,43} = 34.3$, $P < 0.0001$, *post hoc* Dunnett). Data are expressed as mean \pm SEM. ** $P < 0.01$ versus saline group. (C and D) Withdrawal threshold to von Frey filament application on POD1 (C; $KW_{4,34} = 24.8$, $P < 0.0001$, *post hoc* Dunn) and on POD2 (D; $KW_{4,34} = 25.9$, $P < 0.0001$, *post hoc* Dunn) after morphine administration. Rats used in D were the same as those used in C. Data are expressed as median and interquartile range (25th–75th percentile). * $P < 0.05$ versus saline group.



In a previous study of TRPV1 receptor blockade in incisional pain, A-425619 completely blocked incision-induced heat hyperalgesia. Also, A-425619 was effective in reducing acute mechanical hyperalgesia after plantar incision.¹⁰ These investigators proposed that TRPV1 receptors in the central nervous system play an important role in pain and suggest that that significant central nervous system penetration is necessary for a TRPV1 antagonist to produce broad-spectrum analgesia against a variety of nocifensive stimuli in several pain models.¹¹ In the current study, AMG0347 exhibits analgesic effects only on heat hyperalgesia after plantar incision even though blockade of central TRPV1 receptors was shown.

Various strategies including TRPV1 knockout mice, immunologic neutralization of TRPV1, and pharmacologic blockage of TRPV1 are usually performed to determine the role of TRPV1 channel in pain-related behaviors. After incision in TRPV1 knockout mice, heat hyperalgesia is blocked, whereas mechanical responses remained unchanged.²⁹ The marked effect of knockout of the TRPV1 receptor on heat hyperalgesia after incision may be related to species differences between rats and mice or the developmental limitations of a knockout strategy.³⁰

Because morphine is the most commonly used drug for treatment of postoperative pain in humans, we ex-

amined the effects of low doses of morphine against postoperative guarding pain behavior, heat, and mechanical hyperalgesia. In the current study, we tested doses much lower than those used in a previous preclinical study¹⁵ but similar to human postoperative pain studies.^{15,31,32} Interestingly, guarding pain behavior is more sensitive to morphine because very low doses of morphine (0.03 mg/kg) decreased the cumulative pain score after incision. Greater doses (0.3 mg/kg and 1 mg/kg) are necessary to inhibit the heat and mechanical hyperalgesia, which is consistent with a previous study showing that 0.3 mg/kg morphine could reverse the decrease in motivated behavior caused by surgery in rats.³³ This suggests that doses in the clinical range, such as 0.1 mg/kg morphine, may influence unprovoked pain in rats. The correlate to unprovoked pain in rats may be pain at rest in humans, which is usually the parameter that is used to titrate morphine in the recovery room.³²

In conclusion, AMG0347 only affected the heat hyperalgesia to a modest degree, but guarding pain behavior and mechanical responses were not influenced. AMG0347 blocked both peripheral and central TRPV1 receptors. The small effect on heat hyperalgesia after incision may not necessarily translate to clinical pain relief after surgery.

The authors thank Alberto R. Subieta, B.S. (Research Assistant, Department of Anesthesia, University of Iowa Hospitals and Clinics, Iowa City, Iowa), for technical assistance.

References

- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D: The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature* 1997; 389:816-24
- Valtschanoff JG, Rustioni A, Guo A, Hwang SJ: Vanilloid receptor VR1 is both presynaptic and postsynaptic in the superficial laminae of the rat dorsal horn. *J Comp Neurol* 2001; 436:225-35
- Guo A, Vulchanova L, Wang J, Li X, Elde R: Immunocytochemical localization of the vanilloid receptor 1 (VR1): Relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. *Eur J Neurosci* 1999; 11:946-58
- Mezey E, Toth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, Guo A, Blumberg PM, Szallasi A: Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proc Natl Acad Sci U S A* 2000; 97:3655-60
- Szabo T, Biro T, Gonzalez AF, Palkovits M, Blumberg PM: Pharmacological characterization of vanilloid receptor located in the brain. *Brain Res Mol Brain Res* 2002; 98:51-7
- Tominaga M, Caterina MJ: Thermosensation and pain. *J Neurobiol* 2004; 61:3-12
- Bevan S, Szolcsanyi J: Sensory neuron-specific actions of capsaicin: Mechanisms and applications. *Trends Pharmacol Sci* 1990; 11:330-3
- Simone DA, Ngeow JY, Putterman GJ, LaMotte RH: Hyperalgesia to heat after intradermal injection of capsaicin. *Brain Res* 1987; 418:201-3
- Gilchrist HD, Allard BL, Simone DA: Enhanced withdrawal responses to heat and mechanical stimuli following intraplantar injection of capsaicin in rats. *Pain* 1996; 67:179-88
- Honore P, Wismer CT, Mikusa J, Zhu CZ, Zhong C, Gauvin DM, Gomtsyan A, El Kouhen R, Lee CH, Marsh K, Sullivan JP, Faltynek CR, Jarvis MF: A-425619 [1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea], a novel transient receptor potential type V1 receptor antagonist, relieves pathophysiological pain associated with inflammation and tissue injury in rats. *J Pharmacol Exp Ther* 2005; 314:410-21
- Cui M, Honore P, Zhong C, Gauvin D, Mikusa J, Hernandez G, Chandran P, Gomtsyan A, Brown B, Bayburt EK, Marsh K, Bianchi B, McDonald H, Niforatos W, Neelands TR, Moreland RB, Decker MW, Lee CH, Sullivan JP, Faltynek CR: TRPV1 receptors in the CNS play a key role in broad-spectrum analgesia of TRPV1 antagonists. *J Neurosci* 2006; 26:9385-93
- 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
- Brennan TJ, Vandermeulen EP, Gebhart GF: Characterization of a rat model of incisional pain. *Pain* 1996; 64:493-501
- Pogatzki EM, Zahn PK, Brennan TJ: Lumbar catheterization of the subarachnoid space with a 32-gauge polyurethane catheter in the rat. *Eur J Pain* 2000; 4:111-3
- Zahn PK, Gysbers D, Brennan TJ: Effect of systemic and intrathecal morphine in a rat model of postoperative pain. *ANESTHESIOLOGY* 1997; 86:1066-77
- Julius D, Basbaum AI: Molecular mechanisms of nociception. *Nature* 2001; 413:203-10
- Zahn PK, Subieta A, Park SS, Brennan TJ: Effect of blockade of nerve growth factor and tumor necrosis factor on pain behaviors after plantar incision. *J Pain* 2004; 5:157-63
- Banik RK, Subieta AR, Wu C, Brennan TJ: Increased nerve growth factor after rat plantar incision contributes to guarding behavior and heat hyperalgesia. *Pain* 2005; 117:68-76
- Wu C, Boustany L, Liang H, Brennan TJ: Nerve growth factor expression after plantar incision in the rat. *ANESTHESIOLOGY* 2007; 107:128-35
- Zhang X, Huang J, McNaughton PA: NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. *Embo J* 2005; 24:4211-23
- Shu X, Mendell LM: Nerve growth factor acutely sensitizes the response of adult rat sensory neurons to capsaicin. *Neurosci Lett* 1999; 274:159-62
- Bonnington JK, McNaughton PA: Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. *J Physiol* 2003; 551:433-46
- Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D: Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature* 2001; 411:957-62
- Steen KH, Reeh PW: Sustained graded pain and hyperalgesia from harmless experimental tissue acidosis in human skin. *Neurosci Lett* 1993; 154:113-6
- Woo YC, Park SS, Subieta AR, Brennan TJ: Changes in tissue pH and temperature after incision indicate acidosis may contribute to postoperative pain. *ANESTHESIOLOGY* 2004; 101:468-75
- Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D: The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998; 21:531-43
- Reeh PW, Petho G: Nociceptor excitation by thermal sensitization: A hypothesis. *Prog Brain Res* 2000; 129:39-50
- Liang YF, Haake B, Reeh PW: Sustained sensitization and recruitment of rat cutaneous nociceptors by bradykinin and a novel theory of its excitatory action. *J Physiol* 2001; 532:229-39
- 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
- Lariviere WR, Chesler EJ, Mogil JS: Transgenic studies of pain and analgesia: Mutation or background genotype? *J Pharmacol Exp Ther* 2001; 297:467-73
- Richmond CE, Bromley LM, Woolf CJ: Preoperative morphine pre-empts postoperative pain. *Lancet* 1993; 342:73-5
- Aubrun F, Langeron O, Quesnel C, Coriat P, Riou B: Relationships between measurement of pain using visual analog score and morphine requirements during postoperative intravenous morphine titration. *ANESTHESIOLOGY* 2003; 98:1415-21
- Martin TJ, Buechler NL, Kahn W, Crews JC, Eisenach JC: Effects of laparotomy on spontaneous exploratory activity and conditioned operant responding in the rat: A model for postoperative pain. *ANESTHESIOLOGY* 2004; 101:191-203