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


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ABSTRACT

The vanilloid receptor 1 (VR1, TRPV1), which is a member of the transient receptor potential (TRP) superfamily, is highly localized on peripheral and central processes of nociceptive afferent fibers. Activation of TRPV1 contributes to the pronociceptive effects of capsaicin, protons, heat, and various endogenous lipid agonists such as anandamide and N-arachidonoyl-dopamine. A-425619 [1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)urea] is a novel potent and selective antagonist at both human and rat TRPV1 receptors. In vivo, A-425619 dose dependently reduced capsaicin-induced mechanical hyperalgesia (ED50/H11005 45/1H9262 mol/kg p.o.). A-425619 was also effective in models of inflammatory pain and postoperative pain. A-425619 potently reduced complete Freund’s adjuvant-induced chronic inflammatory pain after oral administration (ED50 = 45 μmol/kg p.o.) and was also effective after either i.t. administration or local injection into the inflamed paw. Furthermore, A-425619 maintained efficacy in the postoperative pain model after twice daily dosing p.o. for 5 days. A-425619 also showed partial efficacy in models of neuropathic pain. A-425619 did not alter motor performance at the highest dose tested (300 μmol/kg p.o.). Taken together, the present data indicate that A-425619, a potent and selective antagonist of TRPV1 receptors, effectively relieves acute and chronic inflammatory pain and postoperative pain.

The vanilloid receptor VR1 or TRPV1 is a nonselective cation channel that is activated by exogenous vanilloid compounds such as capsaicin (Caterina and Julius, 2001). Anatomical and functional studies have shown that TRPV1 receptors are expressed on peripheral nociceptors (for review, see Cortright and Szallasi, 2004). Recently, the analgesic potential of TRPV1 receptor blockade has been demonstrated by various approaches including gene disruption, neutralizing antibodies, or receptor antagonism (Caterina et al., 2000; Davis et al., 2000; Kamei et al., 2001; Walker et al., 2003). Although TRPV1 gene-disrupted mice showed mostly normal behavioral responses to noxious heat, they did not develop thermal hyperalgesia to mustard oil or complete Freund’s adjuvant (CFA; Caterina et al., 2000). These results suggest that TRPV1 receptors are required for responses to noxious thermal stimuli under inflammatory conditions but that other mechanisms are in part responsible for normal sensation of noxious heat. Consistent with this conclusion, Davis et al. (2000) showed that TRPV1 knockout mice did not develop thermal hyperalgesia in response to carrageenan but showed normal responses to noxious heat. However, TRPV1 knockout mice did develop mechanical allodynia in response to CFA and mustard oil, showed normal responses to formalin, and developed both thermal hyperalgesia and mechanical allodynia after partial nerve injury (Caterina et al., 2000). A role for TRPV1 in thermal hypersensitivity has also been described in diabetic mice. Following i.t. administration of a TRPV1-neutralizing antibody, a partial reduction in thermal hypersensitivity was observed (Kamei et al., 2001). The effects of capsazepine, a moderately potent TRPV1 receptor antagonist, on pain transmission have been exten-
sively evaluated. Although s.c. injection of capsazepine effectively blocked capsaicin-induced mechanical hyperalgesia in mice, rats, and guinea pigs, it only partially blocked mechanical hyperalgesia in models of inflammatory and neuropathic pain in the guinea pig and had no effect in the rat or the mouse (Walker et al., 2003). However, capsazepine is not pharmacologically selective, limiting its utility for investigating the role of TRPV1 activation in pain transmission (for references, see Wahl et al., 2001). More recently, N-(4-tertiarybutylphenyl)-4-(3-cholorpyridin-2-yl)tetrahydropryazine-1(2H)-oxo-carbox-amide, another TRPV1 receptor antagonist, was shown to have in vivo efficacy in chronic pain models, further validating the potential for TRPV1 receptor antagonists to treat chronic pain (Pomonis et al., 2003).

The present study was undertaken to characterize the antinociceptive effects of A-425619 (Gomtsyan et al., 2005), a novel TRPV1 receptor antagonist. In vitro characterization of A-425619 has shown that this compound potently inhibits (IC$_{50}$ = 2–5 nM) human TRPV1 activation by a variety of stimuli, including capsaicin, acid (pH 5.5), heat (38°C), and endogenous ligands such as anandamide (El Kouhen et al., 2005, companion manuscript). Similarly, A-425619 potently (IC$_{50}$ = 9 nM) blocks capsaicin-evoked currents in rat DRG neurons. In the present study, we report that systemic administration of A-425619 dose dependently reduced nociception in capsaicin-induced mechanical hyperalgesia and in models of acute and chronic inflammatory pain, postoperative pain, and osteoarthritic pain.

Materials and Methods

Subjects. Male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) weighing 200 to 300 g were utilized in most experiments. The hot plate assay was conducted using male CD1 mice weighing 20 to 25 g (Charles River Laboratories). Animals were group housed in Association for Assessment and Accreditation of Laboratory Animal Care-approved facilities at Abbott Laboratories (Abbott, IL) in a temperature-regulated environment with lights on between 7:00 AM and 8:00 PM. Food and water were available ad libitum except during testing. All animal handling and experimental protocols were approved by an Institutional Animal Care and Use Committee. All experiments were performed during the light cycle.

Acute Thermal Nociception. The response to acute thermal stimulation was determined using a commercially available paw thermal stimulator (UARDG; University of California, San Diego, CA). Rats were placed individually in Plexiglas cubicles mounted on a glass surface maintained at 30°C and allowed a 30-min habituation period. A thermal stimulus, in the form of radiant heat emitted from a glass surface maintained at 30°C and allowed a 30-min habituation period, was applied to the plantar surface of each hind paw. In each test session, each rat was tested in three sequential trials at approximately 5-min intervals. Paw withdrawal latencies (PWLs) were calculated as the mean of the two shortest latencies. An assay cut off was set at 20.5 s. A-425619 was injected i.p. 30 min before testing for acute thermal pain.

Hot Plate Assay. Analgesia was measured using an automated hot plate analgesia monitor (model no. AHP16AN; Omnitech Electronics, Columbus, OH). Mice were placed in individual plastic enclosures on the hot plate maintained at 55°C, and the latency until the 10th jump was recorded by disruption of a photocell beam located 12.5 cm above the surface of the hot plate. Mice were removed from the hot plate after either 10 jumps were made or 180 s (test termination) had elapsed, whichever occurred first. The latency until the 10th jump was used for statistical analysis. A-425619 was injected i.p. 30 min before testing for acute thermal pain.

Capsaicin-Induced Mechanical Hyperalgesia. Following a 30-min acclimation period to individual observation cages, 2 μg/10 μl of capsaicin solution was injected s.c. into the plantar aspect of the right hind paw, and the rats were then returned to the clear observation cages for 30 min. The response to mechanical stimulation was then determined by measuring paw withdrawal threshold (PWT) to pressure using the Ugo Basile analgesimeter (Ugo Basile, Comerio, Italy). The animals were gently restrained, and steadily increasing pressure was applied to the dorsal surface of a hind paw via a dome-shaped plastic tip (diameter = 1 mm). The pressure required to elicit paw withdrawal was determined. Two measurements were taken, and the mean was calculated.

Capsaicin-induced mechanical hyperalgesia model was used to demonstrate that in vivo, A-425619 blocks TRPV1 receptor activation by capsaicin. In the first experiment, A-425619 was injected into the paw 15 min before the injection of capsaicin. In the second experiment, A-425619 was injected orally 60 min before the intraplantar injection of capsaicin. Using a pretreatment paradigm (TRPV1 antagonist before capsaicin) allowed for the evaluation of the competition of A-425619 and capsaicin for the TRPV1 receptor in vivo.

Formalin-Induced Spontaneous Pain. Following a 30-min acclimation period to individual observation cages, 50 μl of a 5% formalin solution was injected s.c. into the dorsal aspect of the right hind paw, and the rats were then returned to the clear observation cages. Rats were observed for periods of time corresponding to phase 1 (0–10 min) and phase 2 (30–50 min) of the formalin test. Nociceptive behaviors were recorded from animals during the session by observing each animal for one 60-s observation period during each 5-min interval. Nociceptive behaviors recorded included flinching, licking, or biting the injected paw. A-425619 was injected i.p. 30 min before the injection of formalin.

Carrageenan- and Complete Freund’s Adjuvant-Induced Thermal Hyperalgesia. Unilateral inflammation was induced by injecting 100 μl of a 1% solution of λ-carrageenan or 150 μl of a 50% solution of CFA (Sigma-Aldrich, St. Louis, MO) in physiological saline into the plantar surface of the right hind paw of the rat. The hyperalgesia to thermal stimulation was determined 2 or 48 h following carrageenan or CFA injection, respectively, using the same apparatus as described above for the noxious acute thermal assay. In addition, in the carrageenan model, the volume of paw edema was measured using water displacement with a plethysmometer (Buxco, Sharon, CT) 2 h following carrageenan injection by submerging the hind paw up to the ankle hairline (approximately 1.5 cm). The volume of water displacement was measured using a transducer and recorded by a computer. A-425619 was injected 90 min following carrageenan injection (i.e., 50 min before testing in inflamed rats). In the CFA experiments, CFA was injected 2 days before testing. On the day of testing, A-425619 was injected 5 min (i.t.), 30 min (intraplantar and i.p.), or 60 min (p.o.) before testing for thermal hyperalgesia.

Spinal Nerve (L5/L6) Ligation Model of Neuropathic Pain. As previously described in detail by Kim and Chung (1992), a 1.5-cm incision was made dorsal to the lumbar-sacral plexus. The paraspinal muscles (left side) were separated from the spinous processes, the L5 and L6 spinal nerves isolated, and tightly ligated with 3-0 silk threads. Following hemostasis, the wound was sutured and coated with antibiotic ointment. The rats were allowed to recover and then placed in a cage with soft bedding for 14 days before behavioral testing for mechanical allodynia. A-425619 was injected i.p. 30 min before testing for mechanical allodynia.

Mechanical (tactile) allodynia was measured using calibrated von Frey filaments (Stoelting, Wood Dale, IL). Briefly, rats were placed into individual Plexiglas containers and allowed to acclimate for 15 to 20 min before testing. Paw withdrawal threshold (PWT$\text{_{vonfrey}}$) was determined by increasing and decreasing stimulus intensity and estimated using a Dixon nonparametric test. Only rats with threshold scores ≤4.5 g were considered allodynic and utilized in compound testing experiments.
Sciatic Nerve Ligation Model of Neuropathic Pain. As previously described in detail by Bennett and Xie (1988), a 1.5-cm incision was made 0.5 cm below the pelvis, and the biceps femoris and the gluteus superficialis (right side) were separated. The sciatic nerve was exposed, isolated, and four loose ligatures (5-0 chronic catgut) with 1-mm spacing were placed around it. The rats were allowed to recover and then placed in a cage with soft bedding for 14 days before behavioral testing for mechanical allodynia as described above. A-425619 was injected i.p. 30 min before testing for mechanical allodynia.

Skin Incision Model of Postoperative Pain. As previously described by Brennan et al. (1996), a 1-cm longitudinal incision was made through the skin and fascia of the plantar aspect of the foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally with origin and insertion of the muscle remaining intact. The skin was then closed with two mattress 5-0 nylon sutures. After surgery, the animals were allowed to recover and housed individually with soft bedding. In these experiments, A-425619 was injected 30 min (i.p.) or 60 min (p.o.) before testing for thermal hyperalgesia or mechanical allodynia. Thermal hyperalgesia and mechanical allodynia were tested either 2 or 24 h following surgery.

For the repeated administration study, vehicle or A-425619 (p.o.) was injected 30 min (i.p.) or 60 min (p.o.) before testing for thermal hyperalgesia or mechanical allodynia. Thermal hyperalgesia and mechanical allodynia were tested either 2 or 24 h following surgery. The same day (day 1), animals received a second injection of vehicle or A-425619 12 h later. On days 2 to 5, animals received an injection of vehicle or A-425619 in the morning (7:00 AM) and were tested 60 min later for mechanical allodynia 2 h following surgery. The same day (day 1), animals received a second injection of vehicle or A-425619 and were tested 60 min later for mechanical allodynia.

Osteoarthritic Pain. Unilateral knee joint osteoarthritis was induced in the rats by a single i.a. injection of sodium monooiodacetate (MIA) (Sigma-Aldrich) (3 mg in 0.5 ml of sterile isotonic saline) into the joint cavity using a 26-gauge needle under light (2–4%) halothane (Halocarbon Laboratories, River Edge, NJ) anesthesia. Following injection, the animals were allowed to recover from the effects of anesthesia before returning them to their cages. Since a previous study by Bove et al. (2003) showed that hind limb weight bearing is reduced ipsilaterally following i.a. injection of MIA, hind limb weight-bearing assessment was carried out at day 4 after MIA injection as described below. Four days following MIA injection, A-425619 was injected i.p. 30 min before testing for weight-bearing difference.

Hind limb weight-bearing difference (WBD) between MIA injected and contralateral (uninjected) side was used as a behavioral measure of pain. Differences in weight bearing on the injected versus contralateral hind limb were assessed following the injection of MIA by placing the animals in an Incapacitance Tester (Linton, Norfolk, UK). The animals were restrained in a clear Plexiglas chamber (6 × 3.5 × 3.7 inches), and their hind limbs were positioned over two force plates (2 × 1.5 inches each) placed side by side to measure the weight borne on each hind limb. The animals were allowed to acclimate for a brief period of time before weight-bearing readings (measured in grams) were recorded. Bilateral hind limb weight bearing, consisting of three trials (3 s/trial) was recorded for each animal and then averaged to give a mean weight-bearing score for both ipsilateral and contralateral side hind limbs.

Locomotor Activity and Rotorod Performance. Locomotor activity was recorded in an open field using photobeam activity monitors (AccuScan Instruments, Inc., Columbus, OH). Rotorod performance was measured using an accelerating Rotorod apparatus (Omnitech Electronics, Inc.). For the Rotorod assay, rats were allowed a 30-min acclimation period in the testing room and then placed on a 9-cm diameter rod that increased in speed from 0 to 20 rpm over a 60-s period. The time required for the rat to fall from the rod was recorded, with a maximum score of 60 s. Each rat was given three training sessions. Locomotor activity and Rotorod performance (latencies to fall from the Rotorod) were determined 30 min (i.p.) or 60 min (p.o.) following A-425619 injection.

Intrathecal Catheter Implantation. Under halothane/oxygen anesthesia, animals were placed into an i.t. stereotaxic instrument. An incision was made from the dorsal surface of the occipital bone to the base of the skull (2 cm). Tissue was displaced with a blunt probe so that the cisternal membrane at the base of the skull was clearly seen. A small incision (<1 cm) was made into this membrane. A custom-made catheter (Marsiil Enterprises, Del Mar, CA) was then inserted through this incision point to the lumbar enlargement (8.5 cm). After the notch rested on the cisternal membrane (indicating the tip was in the lumbar enlargement), the external portion of the catheter was threaded into an 18-gauge needle and pulled through the skin. The incision was then closed with surgical staples. The animals were then placed on a heated surface and monitored for recovery. If limb impairment was noticed, the animals were euthanized. Animals were allowed to recover at least 7 days before testing.

Compounds. A-425619 was synthesized at Abbott Laboratories (Gomtsyan et al., 2005). A-425619 was dissolved in 30% polyethylene glycol in water for i.p., oral (p.o.), and intraplantar administration using a volume of 5 ml/kg (i.p. and p.o.) and 50 ml for intraplantar. For i.t. administration, A-425619 was dissolved in 10% di-methylsulfoxide/90% hydroxy-β-cyclodextrin and injected in a volume of 10 μl followed by a saline flush.

Statistics. Analysis of the in vivo data was carried out using analysis of variance. In addition, for the analysis of the time course of effects of A-425619 in the CFA model, repeated analysis of variance was performed. Where appropriate, Fisher’s protected least significant difference was used for post hoc analysis. The level of significance was set at p < 0.05. ED₅₀ values were estimated using least-squares linear regression. Data are presented as mean ± S.E.M.

Results

Pharmacokinetic Profile of A-425619. The pharmacokinetic profile of A-425619 in rats (Fig. 1) was characterized by low plasma clearance (CLp = 0.6 l/h/kg), moderate oral bioavailability (F = 46%), a low volume of distribution (Vₐ = 0.6 l/kg), and a short plasma elimination half-life (t½ = 0.6 h i.v.). Cmax and Tmax following oral dosing at 10 μmol/kg were 1.6 μg/ml and 0.7 h, respectively. In addition, A-425619 had good bioavailability (F = 83%) following i.p. administration.
at 10 μmol/kg with a $C_{\text{max}}$ of 3.0 μg/ml and a $T_{\text{max}}$ of 0.33 h. Plasma and spinal cord samples were harvested 30 min following i.p. administration. Mean plasma levels of A-425619 were 3.28 ± 0.52 μg/ml at 10 μmol/kg and 25.92 ± 2.30 μg/ml at 100 μmol/kg, and spinal cord levels were 0.18 ± 0.02 μg/g at 10 μmol/kg and 1.34 ± 0.34 μg/g at 100 μmol/kg. Thus, the spinal cord to plasma ratio was approximately 5% at both a low and high dose. A-425619 plasma protein binding was 97.1% in rats and 98.0% in humans (Gomtsyan et al., 2005).

**Capsaicin-Induced Mechanical Hyperalgesia.** Local injection of A-425619 into the paw dose dependently and fully blocked capsaicin-induced mechanical hyperalgesia (Fig. 2). Following capsaicin injection, PWT to pressure was significantly reduced (242.2 ± 47.33 g) compared with PWT of rats receiving vehicle injection into the paw (381.0 ± 56.9 g; $p < 0.01$), demonstrating the development of mechanical hyperalgesia. A-425619 injected locally at the site of capsaicin receiving vehicle injection into the paw (381.0 ± 56.9 g; $p < 0.01$), demonstrating the development of mechanical hyperalgesia. A-425619 injected locally at the site of capsaicin injection dose dependently increased PWT in the capsaicin-algesia. A-425619 injected locally at the site of capsaicin injection dose dependently increased PWT in the capsaicin-induced mechanical hyperalgesia (Fig. 2). A-425619 was fully efficacious at 300 nmol ($p < 0.01$) with an ED$_{50}$ of 48 nmol/rat. A-425619 (300 nmol) injection into the contralateral paw had no effect on PWT of the capsaicin-injected paw (PWT, 219.0 ± 28.74 g).

Oral administration of A-425619 also prevented capsaicin-induced mechanical hyperalgesia (Fig. 3). As in the previous experiment, capsaicin injection significantly reduced PWT to pressure (177.0 ± 24.12 g) compared with PWT of rats receiving vehicle injection into the paw (347.0 ± 54.9 g; $p < 0.01$). A-425619 following oral administration fully prevented capsaicin-induced mechanical hyperalgesia with an ED$_{50}$ of 45 μmol/kg p.o. and showed full efficacy at 100 μmol/kg p.o. (Fig. 3).

**CFA-Induced Chronic Inflammatory Thermal Hyperalgesia.** CFA injection into the hind paw induced a significant decrease in PWL to thermal stimulation 48 h following CFA injection (PWL control, 11.4 ± 0.7 s versus PWL inflamed, 4.9 ± 0.6 s, $p < 0.01$), demonstrating the development of thermal hyperalgesia (Fig. 4A, see also Fig. 4B). A-425619 dose dependently and fully relieved CFA-induced thermal hyperalgesia after i.p. (ED$_{50}$ 51 μmol/kg; Fig. 4A) or p.o. (ED$_{50}$ 40 μmol/kg; Fig. 4B) administration. Under the same conditions, A-425619 had no effect on PWL of the contralateral noninflamed paw, indicative of a specific antihyperalgesic effect in this model.

The antinociceptive effects of A-425619 (100 μmol/kg) in the CFA model were rapid in onset and long-lasting (Fig. 5). Significant antinociception was observed at 15 min following i.p. administration and persisted for at least 8 h. At 24 h following A-425619 administration, PWL were back to hyperalgesic control values ($p > 0.05$; Fig. 5).

To investigate potential site(s) of action for A-425619 in inflammatory pain, local administration into the paw or i.t. injection studies were performed. A-425619 (300 nmol) produced a 56.2 ± 4.6% decrease in thermal hyperalgesia following intraplantar injection into the inflamed paw ($p < 0.05$; Fig. 6A). A-425619 (300 nmol) injection into the contralateral paw (vehicle/A-425619 group) had only a weak antihyperalgesic effect (20.3 ± 4.6% effect, $p < 0.05$) that was significantly smaller than the effects observed when A-425619 was directly injected into the inflamed paw ($p < 0.05$). This dose of A-425619 had no effect on the PWL of the contralateral control paw when injected into either the inflamed or contralateral paw (Fig. 6A). In addition, following i.t. administration, A-425619 reduced CFA-induced thermal hyperalgesia by 63.0 ± 5.8% at 100 nmol ($p < 0.05$; Fig. 6B). This dose of A-425619 injected i.t. had no effect on the PWL of the contralateral control paw (Fig. 6B).

![Fig. 2. Antinoceceptive effects of A-425619 following intraplantar administration on capsaicin-induced mechanical hyperalgesia.](image-url)
Carrageenan-Induced Acute Inflammatory Thermal Hyperalgesia. Carrageenan injection into the hind paw induced a significant decrease in PWL to thermal stimulation (PWL control, 9.1 ± 0.3 s versus PWL inflamed, 3.1 ± 0.3 s, p < 0.01), demonstrating the development of thermal hyperalgesia 2 h following carrageenan injection (Fig. 7). A-425619 dose dependently relieved carrageenan-induced thermal hyperalgesia with an ED50 of 50 μmol/kg i.p. and 77.8% ± 10.0% effect at the highest dose tested (Fig. 7). Under the same conditions, A-425619 had no effect on PWL of the contralateral noninflamed paw, indicative of a specific antihyperalgesic effect in this model.

Carrageenan-Induced Paw Edema. The acute anti-inflammatory effects of A-425619 were assessed in the carrageenan-induced paw edema model. A-425619 (10–100 μmol/kg i.p.) did not significantly decrease carrageenan-induced paw edema [F(3,20) = 1.423, p > 0.05]. At 100 μmol/kg, A-425619 induced 34.6 ± 16.7% decrease in paw volume (0.8 ± 0.2...
versus 1.2 ± 0.1 ml in vehicle-treated animals, $p > 0.05$; Table 1).

**Skin Incision-Induced Thermal Hyperalgesia.** Surgery of the plantar surface of the rat's hind paw induced a decrease in PWL to thermal stimulation 2 h (PWL control, 11.0 ± 0.8 s; PWL injured, 3.1 ± 0.5 s, $p < 0.01$) and 24 h (PWL control, 11.2 ± 0.5 s; PWL injured, 3.1 ± 0.2 s, $p < 0.01$) after surgery (Figs. 8 and 9), demonstrating the development of thermal hyperalgesia. A-425619 dose dependently and fully relieved skin incision-induced acute thermal hyperalgesia. Following i.p. administration, A-425619 had an ED$_{50}$ of 65 nmol/kg (Fig. 8A). In addition, A-425619 was orally active at reducing skin incision-induced thermal hyperalgesia (ED$_{50}$, 80 nmol/kg p.o.; Fig. 8B). A-425619 produced an
88.5 ± 6.4% decrease in thermal hyperalgesia at 300 μmol/kg p.o. (PWL injured, 11.3 ± 1.7 s, p < 0.01 versus vehicle-treated animals, PWL injured, 3.1 ± 0.3 s and PWL control, 11.7 ± 0.3 s). Under the same conditions, A-425619 had no effect on PWL of the contralateral noninjured paw, indicative of a specific antihyperalgesic effect in this model.

A-425619 also dose dependently relieved skin incision-induced thermal hyperalgesia observed 24 h postsurgery following i.p. administration (Fig. 9). A-425619 produced a 44.2 ± 2.9% decrease in thermal hyperalgesia at 100 μmol/kg i.p. (p < 0.01). Under the same conditions, A-425619 had no effect on PWL of the contralateral noninjured paw, indicative of a specific antihyperalgesic effect in this model.

**Skin Incision-Induced Mechanical Allodynia.** As was seen for thermal hyperalgesia, surgery of the plantar surface of the rat’s hind paw induced a decrease in PWT_vonfrey to mechanical stimulation with von Frey monofilaments 2 (PWT_vonfrey control, 12.1 ± 0.5 g; PWT_vonfrey injured, 1.4 ± 0.1 g, p < 0.01) and 24 (PWT_vonfrey control, 13.8 ± 0.3 g; PWT_vonfrey injured, 1.0 ± 0.1 g, p < 0.01) h after surgery, demonstrating the development of mechanical allodynia (Brennan et al., 1996). A-425619 was weakly active in reducing skin incision-induced acute mechanical allodynia \[^{[F(3,20) = 4.149, p < 0.02]}\]. A-425619 reduced mechanical allodynia only by 18.9 ± 6.2% at 100 μmol/kg i.p. (p < 0.01, Table 1). A-425619 showed better efficacy on mechanical allodynia 24 h following surgery \[^{[F(3,20) = 309.042, p < 0.0001]}\] with a 42.2 ± 6.2% reduction of mechanical allodynia at 100 μmol/kg i.p. (p < 0.01, Table 1; see also 24-h time point, day 2, Fig. 10).

The antinociceptive effects of A-425619 (100 μmol/kg p.o.) were sustained after chronic dosing twice daily for 5 days. As

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**TABLE 1**

<table>
<thead>
<tr>
<th>Pain Model</th>
<th>ED50 (μmol/kg i.p.)</th>
<th>Effect at 100 μmol/kg i.p.</th>
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<tr>
<td>Mouse hotplate</td>
<td>&gt;100</td>
<td>9 ± 1</td>
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<td>Inflammatory pain</td>
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<td>64 ± 15*</td>
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<td>Weight-bearing difference</td>
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* Significantly different (p < 0.05) from vehicle-treated animals (n = 6–12 per group).
Fig. 8. Antinociceptive effects of A-425619 in the skin incision model of postoperative pain, testing for thermal hyperalgesia 2 h after surgery following i.p. [A; F(7,40) = 35.452, p < 0.0001] and p.o. [B; F(9,56) = 53.635, p < 0.0001] administration. In these experiments, following surgery, A-425619 was injected 30 min (i.p.) or 60 min (p.o.) before testing. Circles, paw withdrawal latencies ipsilateral to the injury; squares, paw withdrawal latencies contralateral to the injury. Data represent mean ± S.E.M. **, p < 0.01 compared with vehicle-treated animals. +++, p < 0.01 compared with incised paw. n = 6 to 12 per group.

Fig. 9. Antinociceptive effects of A-425619 (i.p.) in the skin incision model of postoperative pain, testing for thermal hyperalgesia 24 h after surgery following i.p. [A; F(7,40) = 460.500, p < 0.0001] and p.o. [B; F(9,56) = 46.800, p < 0.0001] administration. In these experiments, following surgery, A-425619 was injected 30 min (i.p.) or 60 min (p.o.) before testing. Circles, paw withdrawal latencies ipsilateral to the injury; squares, paw withdrawal latencies contralateral to the injury. Data represent mean ± S.E.M. *, p < 0.05; **, p < 0.01 compared with vehicle-treated animals. +, p < 0.01 compared with incised paw. n = 6 per group.

MIA-Induced Osteoarthritic Pain. MIA injection into the knee joint induces osteoarthritis of the joint associated with weight-bearing differences between the injured and noninjured hind limbs (Bove et al., 2003). Four days after MIA injection, substantial WBD was observed between the injured and noninjured hind limbs (48.1 ± 4.9 g; Fig. 11). A-425619 significantly decreased MIA-induced increase in WBD with 24.7 ± 5.5% effect at 100 μmol/kg and 46.8 ± 5.5% effect at 300 μmol/kg i.p. (p < 0.01 for both; Fig. 11).

Spinal Nerve Injury-Induced Mechanical Allodynia. Spinal nerve injury (L5-L6 spinal nerve ligation, Chung model) produced a decrease in PWT<sub>von</sub> to mechanical stimulation with von Frey monofilaments 2 weeks following injury (PWT<sub>von</sub> control, 13.7 ± 0.3 g; PWT<sub>von</sub> injured, 2.8 ± 0.2 g, p < 0.01), demonstrating the development of mechanical allodynia. A-425619 was weak at reducing spinal nerve injury-induced mechanical allodynia [F(3,20) = 16.065, p < 0.0001]. A-425619 only induced a 35.9 ± 4.7% reduction at 100 μmol/kg i.p. (PWT<sub>von</sub> injured, 6.7 ± 0.5 g, p < 0.01; Table 1).

Sciatic Nerve Injury-Induced Mechanical Allodynia. Chronic constriction injury of the sciatic nerve (Bennett model) produced a decrease in PWT<sub>von</sub> to mechanical stimulation with von Frey monofilaments 2 weeks following surgery (PWT<sub>von</sub> control, 11.3 ± 0.6 g; PWT<sub>von</sub> injured, 2.3 ± 0.2 g, p < 0.01), demonstrating the development of mechanical allodynia. A-425619 was weak in reducing sciatic nerve injury-induced mechanical allodynia [F(3,20) = 15.172, p < 0.0001]. A-425619 produced a 36.1 ± 4.4% reduction at 100 μmol/kg i.p. (PWT<sub>von</sub> injured, 5.5 ± 0.4 g, p < 0.01; Table 1). Intrathecal injection of A-425619 was also found to significantly reduce mechanical allodynia (32.9 ± 9.6% reduction at 300 nmol, p < 0.01) in this model.

Acute Thermal Pain. A-425619 did not show any significant effects on acute thermal pain in naive rats or naive CD1 mice. In the rat, A-425619-treated animals (100 μmol/kg i.p.) had a PWL to acute thermal stimulation of 8.6 ± 0.4 s.
compared with 8.2 ± 0.3 in vehicle-treated animals (p > 0.05, Table 1). In the mouse hot plate assay, A-425619-treated animals (100 μmol/kg i.p.) had a latency to jump of 81.6 ± 8.1 s compared with 71.2 ± 4.7 in vehicle-treated animals (p > 0.05, Table 1).

Formalin-Induced Spontaneous Pain. In the rat formalin assay, 5% formalin was injected into the right hind paw to induce a characteristic biphasic response. A-425619 did not show any significant effects on formalin-induced spontaneous paw flinching [$F(3,19) = 1.410$, p > 0.05]. The number of formalin-induced flinches was not significantly different between vehicle-treated animals (67.3 ± 5.8 flinches) and A-425619-treated animals (58.3 ± 3.3 flinches at 100 μmol/kg i.p., Table 1).

Effects on Motor Activity and General CNS Function. A-425619 had no significant effect on motor coordination at doses up to 100 μmol/kg i.p. [$F(3,28) = 2.84$, p > 0.05] and 300 μmol/kg p.o. [$F(3,28) = 1.13$, p > 0.05], as measured
by the ability of rats to run on an accelerating rotating rod (control latency = 59.3 ± 0.5 s). A-425619 also had no significant effect on spontaneous exploratory activity at doses up to 100 μmol/kg i.p. [F(3,28) = 1.28, p > 0.05] and 300 μmol/kg p.o. [F(3,28) = 0.64, p > 0.05], as measured by the rat total horizontal activity assessed in a novel open field (control = 6882.4 ± 493.9). Rats were fully awake, responsive to stimuli, and retained the righting reflex, consistent with their ability to perform the Rotorod test at all doses tested.

Discussion

A-425619, a novel, potent, and selective TRPV1 receptor antagonist, dose dependently blocked capsaicin-induced nociception and was most potent and efficacious in animal pain models associated with low pH such as acute and chronic inflammation and postoperative pain. The TRPV1 receptor has been shown to be a molecular integrator of various nociceptive stimuli, such as increased temperature, acidic conditions, and various endogenous lipids that are present under inflammatory conditions. Specifically, it has recently been shown that endovanilloids, including anandamide (Dinis et al., 2004), leukotriene B4 (Ferreira et al., 2004), and 12-hydroperoxyeicosatetraenoic acid (Shin et al., 2002) are produced under inflammatory conditions. Although these various agonists can directly activate the TRPV1 receptor, they can also sensitize TRPV1 to the effects of other activators (Tominaga et al., 1998, 2001; Caterina and Julius, 2001; Di Marzo et al., 2002). Furthermore, it has been shown that substances produced by local inflammation such as ATP, bradykinin, and nerve growth factor can increase capsaicin-evoked currents in TRPV1-expressing neurons (Tominaga et al., 2001; Di Marzo et al., 2002). These findings, together with the analgesic profile of A-425619, provide further evidence that TRPV1 receptor activation plays a greater role in inflammatory pain than in normal nociception.

The present data show that A-425619 completely blocked capsaicin-induced mechanical hyperalgesia when injected systemically or locally at the site of capsaicin injection. These results demonstrate that A-425619 can block, in a dose-related manner, the direct activation of nociceptors by capsaicin and confirm that in vivo, A-425619 behaves as an orally active TRPV1 receptor antagonist. In agreement with these results, A-425619 was shown in vitro to be a competitive antagonist of TRPV1 receptor activation by capsaicin (El Kouhen et al., 2005, companion paper).

TRPV1 gene-disrupted mice are insensitive to capsaicin-evoked nociception and also show reduced inflammatory hyperalgesia (Caterina et al., 2000; Davis et al., 2000). The present data show that systemic administration of the TRPV1-selective antagonist, A-425619, fully reversed thermal hyperalgesia in both acute (carrageenan) and chronic (CFA) inflammatory pain models. These antihyperalgesic actions were also characterized by a rapid onset and prolonged duration of action. Consistent with a selective antihyperalgesic, rather than analgesic effect, A-425619 lacked analgesic efficacy in noninflammatory thermal pain models. Thus, the in vivo behavioral effects of A-425619 are in close agreement with the phenotype observed in TRPV1 knockout mice when challenged with inflammatory agents such as carrageenan, mustard oil, or CFA (Caterina et al., 2000; Davis et al., 2000).

Interestingly, although systemic A-425619 completely blocked CFA-induced thermal hyperalgesia, systemic A-425619 had no effect on formalin-induced persistent spontaneous flinching behavior. Since the second phase of the formalin response is believed to be due, primarily, to central sensitization (Haley et al., 1990), these data suggest that A-425619 preferentially decreases peripheral sensitization rather than central sensitization. This lack of effect of A-425619 in the formalin model could be associated with the relatively low CNS penetration by this compound (spinal levels are 5% of plasma levels for both the 10 and 100 μmol/kg doses). However, the lack of effect of A-425619 in the formalin model is in agreement with the lack of changes in the formalin response in TRPV1 knockout compared with wild-type mice (Caterina et al., 2000). Also in agreement with the TRPV1 knockout mouse phenotype, systemic injection of A-425619 was only weakly effective at reducing mechanical allodynia in two models of nerve injury-induced neuropathic pain. Taken together, these results demonstrate that blockade of TRPV1 receptor activation, by gene knockout or by a selective receptor antagonist, is most effective in reducing inflammatory hyperalgesia.

Studies of TRPV1 receptor regulation have shown that expression is increased in nerve fibers innervating the skin during inflammation (Carlton and Coggeshall, 2001) and that there is increased axonal transport of TRPV1 receptors toward the site of injury (Tohda et al., 2001). Additionally, peripheral inflammation is also associated with increased TRPV1 receptor expression in dorsal root ganglion neurons and in the superficial layers of the spinal cord (Luo et al., 2004). In agreement with these findings, A-425619 effectively reduced inflammatory hyperalgesia following both i.t. infusion and local injection into the inflamed paw. The peripheral analgesic effects of A-425619 were primarily localized to the inflamed paw, rather than systemically mediated, since local administration of A-425619 into the contralateral noninflamed paw produced only modest antinociception. The receptor regulation data and the analgesic effects of A-425619 indicate that TRPV1 receptors on both peripheral and central terminals of sensory afferents contribute to inflammatory hyperalgesia.

In contrast to inflammatory pain states, TRPV1 receptor expression is decreased in injured DRG neurons in various models of nerve injury as shown by in situ hybridization, immunohistochemistry, and Western blot (Rashid et al., 2003a,b). However, in these same models, TRPV1 expression was reported to be unaltered or increased in uninjured neurons (Michael and Priestley, 1999; Hudson et al., 2001; Fukuoka et al., 2002; Rashid et al., 2003a,b). The present data show that A-425619 had only weak efficacy in two models of neuropathic pain, which is likely due, at least in part, to the inability of A-425619 to significantly alter central sensitization because of its relatively low access to the CNS after systemic administration. This hypothesis is supported by the ability of i.t. delivered A-425619 to reduce sciatic nerve injury-induced mechanical allodynia. In addition, Gillen et al. (2004) have shown that i.t. administration of TRPV1 antisense reduces mechanical allodynia observed after spinal nerve ligation and that i.t. TRPV1 siRNA decreases cold allodynia associated with sciatic nerve injury. Skin incision-induced thermal hyperalgesia and mechanical allodynia in the rat are considered to model postoperative pain in humans (Brennan et al., 1996). In this model,
A-425619 effectively decreased both acute and persistent thermal hyperalgesia following surgery but showed weaker activity at decreasing mechanical allodynia. Consistent with these effects, both A-δ fibers and C fibers express the TRPV1 receptor (Michael and Priestley, 1999; Valtchanoff et al., 2001) and become sensitized after skin incision (Pogatzki et al., 2002). Interestingly, Woo et al. (2004) have recently shown that a local decrease in pH occurs immediately after skin incision that persists for at least 4 days. These changes were highly correlated with the changes in nociceptive sensitivity. A-425619 also dose dependently decreased osteoarthritic pain as measured by an improvement in weight-bearing difference between the injured and uninjured hind limbs in MIA-induced osteoarthritic rats. Although topical capsaicin has been shown to decrease osteoarthritic pain in the clinic when applied to the affected joint (for review, see McCleane, 2000), this is the first demonstration of the antinoceptive effects of a selective TRPV1 receptor antagonist in an osteoarthritis pain model. Rich peptidergic fibers have been shown to innervate both the bone itself and the periosteum around the bone (Irie et al., 2002; Mach et al., 2002). In addition, a recent study by Cho and Valtchanoff (2004) showed increased TRPV1 expression in the peripheral axons of DRG neurons in the joint capsule of osteoarthritic rats, consistent with the efficacy of A-425619 in this model. Furthermore, a decrease in pH at the site of bone resorption has been well characterized, further supporting a role for TRPV1 activation in osteoarthritic pain (Baron et al., 1985; Blair et al., 1989).

Several novel vanilloid such as 6-iodo-nor-di-hydro-capsaicin (Appendino et al., 2003) and nonvanilloid TRPV1 receptor antagonists such as SB-366791 (Guntherho et al., 2004), compound 41 (Swanson et al., 2005), and AMG9810 (Doherty et al., 2005; Gavva et al., 2005) have been identified as potent (15–100 nM) at recombinant hTRPV1 TRPV1 receptor antagonists. Intraportal administration of compound 41 and AMG9810 has been reported to block the algogenic effects of capsaicin in rats and AMG9810-reduced CFA-induced inflammatory hyperalgesia (Gavva et al., 2005). The present data demonstrate that A-425619 is a novel, highly potent, and selective TRPV1 receptor antagonist that is orally effective at relieving nociception in a spectrum of well characterized animal models of pain, including acute and chronic inflammatory pain, postoperative pain, and pain associated with osteoarthritis. The evaluation of A-425619 in these diverse pain models also significantly expands the antinoceptive profile for selective TRPV1 antagonists. These effects were observed in the absence of any locomotor impairment, and A-425619 maintained its antinoceptive efficacy after chronic dosing. A-425619, and other highly selective TRPV1 antagonists, will be useful in the further characterization of the role of TRPV1 receptors in various nociceptive conditions. Taken together, the preclinical profile of A-425619 indicates that TRPV1 antagonists may be particularly effective in the clinical alleviation of nociceptive pain associated with persistent inflammation including arthritis and postsurgical pain.

References


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