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# A-425619, a Novel TRPV1 Receptor Antagonist, Relieves Pathophysiological Pain Associated with Inflammation and Tissue Injury in Rats

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**Running Title:** Analgesic profile of the TRPV1 antagonist A-425619

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Abbreviations: CFA: complete Freund's adjuvant; Cmax: maximum concentration; FPLSD: Fisher's Protected Least Significant Difference; MIA: monoiodoacetate; NADA: N-arachidonoyl-dopamine; OA: osteoarthritis; PWL: paw withdrawal latency; PWT: paw withdrawal threshold; Tmax: time of peak concentration; TRPV1: transient receptor potential type V1

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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 (NADA). A-425619 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 ($ED_{50}$ = 45 µmol/kg, p.o.). A-425619 was also effective in models of inflammatory pain and post-operative pain. A-425619 potently reduced complete Freund's adjuvant (CFA)-induced chronic inflammatory pain after oral administration ($ED_{50}$ = 40 µmol/kg, p.o.) and was also effective after either intrathecal administration or local injection into the inflamed paw. Furthermore, A-425619 maintained efficacy in the post-operative pain model after twice daily dosing p.o. for five 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 post-operative pain.

## Introduction

The vanilloid receptor VR1 or TRPV1 is a non-selective 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 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 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 knock out mice did not develop thermal hyperalgesia in response to carrageenan, but showed normal responses to noxious heat. However, TRPV1 knock out 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 intrathecal 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 extensively evaluated. Although subcutaneous 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 (references in Wahl et al., 2001). More recently, BCTC (N-(4-Tertiarybutylphenyl)-4-(3-cholorphyridin-2-yl)tetrahydropryazine-1(2H0-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 (1-Isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea, Figure 1; 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, post-operative pain, and osteoarthritic pain.

## Methods

**Subjects.** Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 200-300 grams were utilized in most experiments. The hotplate assay was conducted using male CD1 mice weighing 20-25 g (Charles River, Wilmington, MA). Animals were group housed in AAALAC approved facilities at Abbott Laboratories in a temperature-regulated environment with lights on between 0700 and 2000 hours. 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 (IACUC). 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 Plexiglass cubicles mounted on a glass surface maintained at 30$^{o}$C, and allowed a 30 min habituation period. A thermal stimulus, in the form of radiant heat emitted from a focused projection bulb, was then applied to the plantar surface of each hind paw. In each test session, each rat was tested in 3 sequential trials at approximately 5 min intervals. Paw withdrawal latencies (PWL) were calculated as the mean of the two shortest latencies. An assay cut off was set at 20.5 seconds. A-425619 was injected i.p. 30 minutes before testing for acute thermal pain.

**Hot-plate assay**. Analgesia was measured using an automated hot-plate analgesia monitor (Model # AHP16AN, Omnitech Electronics, Columbus, OH).

Mice were place in individual plastic enclosures on the hotplate maintained at 55ºC and the latency until the tenth 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 hotplate after either ten jumps were made or 180 sec (test termination) had elapsed, whichever occurred first. The latency until the tenth jump was used for statistical analysis. A-425619 was injected i.p. 30 minutes 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 subcutaneously (s.c.) into the plantar aspect of the right hind paw and the rats were then returned to the clear observation cages for 30 minutes. The response to mechanical stimulation was then determined by measuring paw withdrawal threshold (PWT) to pressure using the Ugo Basile analgesymeter (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 minutes before the injection of capsaicin. In the second experiment, A-425619 was injected orally 60 minutes 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-sec 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 minutes before the injection of formalin.

**Carrageenan- and Complete Freund's Adjuvant-induced Thermal Hyperalgesia.** Unilateral inflammation was induced by injecting 100µl of a 1% solution of λ-carrageenan or 150µl of a 50% solution of complete Freund's adjuvant (CFA) (Sigma Chemical Co., 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 2hr or 48hr 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) 2hr following carrageenan injection, by submerging the hind paw up

to the ankle hairline (approximately 1.5 cm). The volume of water displacement was measured by a transducer and recorded by a computer. A-425619 was injected 90 minutes following carrageenan injection (i.e. 30 minutes 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 minutes (intrathecal), 30 minutes (intraplantar and i.p.), or 60 minutes (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 lumbosacral 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 minutes 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 plexiglass containers and allowed to acclimate for 15-20 minutes before testing. Paw withdrawal threshold ($PWT_{vonfrey}$) was determined by increasing and decreasing stimulus intensity and estimated using a Dixon non-parametric 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 0.5cm below the pelvis and the biceps femoris and the gluteous superficialis (right side) were separated. The sciatic nerve was exposed, isolated, and four loose ligatures (5-0 chromic 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 minutes before testing for mechanical allodynia.

**Skin-incision Model of Post-operative 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 2 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 minutes (i.p.) or 60 minutes (p.o.) before testing for thermal hyperalgesia or mechanical allodynia. Thermal hyperalgesia and mechanical allodynia were tested either 2 hours or 24 hours following surgery. For the repeated administration study, vehicle or A-425619 (p.o.) was injected 1 hour following surgery and animals were tested for mechanical allodynia 2 hours following surgery. The same day (day 1), animals received a second injection of vehicle or A-425619 12 hours later. On days 2-5, animals received an injection of vehicle or A-425619 in the

morning (7:00 am) and were tested 60 minutes later for mechanical allodynia. On days 2-4, animals received a second injection 12 hours later. As stated above, on day 5, animals received one injection of vehicle or A-425619 and were tested 60 minutes later for mechanical allodynia.

**Osteoarthritic Pain.** Unilateral knee joint osteoarthritis was induced in the rats by a single intra-articular (i.a.) injection of sodium monoiodoacetate (MIA) (Sigma-Aldrich, St. Louis, MO) (3 mg in 0.05 ml sterile isotonic saline) into the joint cavity using a 26G 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 minutes 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 plexiglass chamber (6”x 3.5”x 3.7”), and their hind limbs were positioned over two force plates (2”x 1.5” 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 3 trials (3 seconds/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, Columbus, OH). Rotorod performance was measured using an accelerating rotorod apparatus (Omnitech Electronics, Inc. Columbus, OH). 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 sec period. The time required for the rat to fall from the rod was recorded, with a maximum score of 60 sec. Each rat was given 3 training sessions. Locomotor activity and rotorod performance (latencies to fall from the rotorod) were determined 30 minutes (i.p.) or 60 minutes (p.o.) following A-425619 injection.

**Intrathecal catheter implantation.** Under halothane/oxygen anesthesia, animals were placed into an intrathecal 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 (Marsil 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 (1-Isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea) was synthesized at Abbott Laboratories (Gomtsyan et al., 2005). A-425619 was dissolved in 30% polyethylene glycol (PEG400) in water for intraperitoneal (i.p.), oral (p.o.), and intraplantar (i.pl.) administration using a volume of 5ml/kg (i.p. and p.o.) and 50 µl for i.pl.. For intrathecal administration, A-425619 was dissolved in 10% di-methylsulfoxide / 90% hydroxy-β-cyclodextrine 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 ANOVA was performed. Where appropriate, Fisher's Protected Least Significant Difference (FPLSD) was used for post-hoc analysis. The level of significance was set at $p < 0.05$. $ED_{50}$ values were estimated using least squares linear regression. Data are presented as mean ± S.E.M..

## Results

**Pharmacokinetic profile of A-425619.** The pharmacokinetic (PK) profile of A-425619 in rats (Figure 1) was characterized by low plasma clearance (CLp = 0.6 L/hr·kg), moderate oral bioavailability (F = 46%), a low volume of distribution ($V_{ß}$ = 0.6 L/kg), and a short plasma elimination half-life ($T_{1/2}$ = 0.6 h, i.v.). Cmax and Tmax following oral dosing at 10 µmol/kg were 1.6µg/ml and 0.7 hours, respectively. In addition, A-425619 had good bioavailability (F = 83%) following intraperitoneal administration at 10 µmol/kg with a Cmax of 3.0µg/ml and a Tmax of 0.33 hours. Plasma and spinal cord samples were harvested 30 minutes following i.p. administration. Mean plasma levels of A-425619 were 3.28 ± 0.52 µg/ml at 10µmol/kg and 25.92 ± 2.30 µg/ml at 100µmol/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 rat and 98.0 % in human (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 (Figure 2). Following capsaicin injection, PWT to pressure was significantly reduced (242.2 ± 47.33 g) compared to 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 injection dose-dependently increased PWT in the capsaicin-injected paw (Figure 2). A-425619 was fully efficacious at 300 nmol ($p < 0.01$) with an

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$ED_{50}$ 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 (Figure 3). As in the previous experiment, capsaicin injection significantly reduced PWT to pressure (177.0 ± 24.12 g) compared to 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 µml/kg, p.o. (Figure 3).

**CFA-induced chronic inflammatory thermal hyperalgesia.** CFA injection into the hind paw induced a significant decrease in PWL to thermal stimulation 48 hours following CFA injection (PWL control: 11.4 ± 0.7 sec vs. PWL inflamed: 4.9 ± 0.6 sec, $p < 0.01$), demonstrating the development of thermal hyperalgesia (Figure 4A, see also Figure 4B). A-425619 dose-dependently and fully relieved CFA-induced thermal hyperalgesia after i.p. ($ED_{50}$ of 51 µmol/kg, Figure 5A) or p.o. ($ED_{50}$ of 40 µmol/kg Figure 5B) administration. Under the same conditions, A-425619 had no effect on PWL of the contralateral non-inflamed 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 (Figure 5). Significant antinociception was observed at 15 minutes following i.p. administration and persisted for at least 8 hours. At 24 hours following A-425619 administration, PWL were back to hyperalgesic control values ($p > 0.05$, Figure 5).

To investigate potential site(s) of action for A-425619 in inflammatory pain, local administration into the paw or intrathecal injection studies were performed. A-425619 (300 nmol) produced 56.2 ± 4.6% decrease in thermal hyperalgesia following intraplantar injection into the inflamed paw ($p < 0.05$, Figure 6A). A-425619 (300 nmol) injection into the contralateral paw (vehicle/A-425619 group) had only a weak anti-hyperalgesic 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 (Figure 6A). In addition, following intrathecal administration, A-425619 reduced CFA-induced thermal hyperalgesia by 63.0 ± 13.0% at 100 nmol ($p < 0.05$, Figure 6B). This dose of A-425619 injected intrathecally had no effect on the PWL of the contralateral control paw (Figure 6B).

**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 sec vs. PWL inflamed: 3.1 ± 0.3 sec, $p < 0.01$), demonstrating the development of thermal hyperalgesia 2 hours following carrageenan injection (Figure 7). A-425619 dose-dependently relieved carrageenan-induced thermal hyperalgesia with an $ED_{50}$ of 50 µmol/kg, i.p. and 77.8% ± 10.0% effect at the highest dose tested (Figure 7). Under the same conditions, A-425619 had no effect on PWL of the contralateral non-inflamed paw, indicative of a specific anti-hyperalgesic 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 ml vs. 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 hours (PWL control 11.0 ± 0.8 sec, PWL injured 3.1 ± 0.5 sec, $p < 0.01$) and 24 hours (PWL control 11.2 ± 0.5 sec, PWL injured 3.1 ± 0.2 sec, $p < 0.01$) after surgery (Figure 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 µmol/kg (Figure 8A). In addition, A-425619 was orally active at reducing skin incision–induced thermal hyperalgesia ($ED_{50}$ of 80 µmol/kg, p.o., Figure 8B). A-425619 produced an 88.5 ± 6.4 % decrease in thermal hyperalgesia at 300µmol/kg, p.o. (PWL injured 11.3 ± 1.7 sec, $p < 0.01$ vs. vehicle-treated animals PWL injured 3.1 ± 0.3 sec and PWL control 11.7 ± 0.3 sec). Under the same conditions, A-425619 had no effect on PWL of the contralateral non-injured paw, indicative of a specific anti-hyperalgesic effect in this model.

A-425619 also dose-dependently relieved skin incision-induced thermal hyperalgesia observed 24 hours post-surgery following i.p. administration (Figure 9). A-425619 produced 44.2 ± 2.9 % decrease in thermal hyperalgesia at

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100µmol/kg, i.p. ($p < 0.01$). Under the same conditions, A-425619 had no effect on PWL of the contralateral non-injured 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 hours ($PWT_{vonfrey}$ control 12.1 ± 0.5 g, $PWT_{vonfrey}$ injured 1.4 ± 0.1 g, $p < 0.01$) and 24 hours ($PWT_{vonfrey}$ control 13.8 ± 0.3 g, $PWT_{vonfrey}$ injured 1.0 ± 0.1 g, $p < 0.01$) 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 hours 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 hrs time point (Day 2) Figure 10).

The antinociceptive effects of A-425619 (100 µmol/kg, p.o.) were sustained after chronic dosing twice daily for 5 days. As seen in Figure 10, A-425619 produced a 42.6 ± 13.8 % effect ($p < 0.01$) in reducing mechanical allodynia when tested at 24 hours following surgery (Day 2) and still produced a 37.6 ± 6.9 % analgesic effect ($p < 0.01$) when tested on day 5 following surgery after 5 days of chronic dosing. Under the same experimental conditions, the

antinociceptive effects of morphine (3mg/kg, s.c.) in this model were greatly reduced (day 1: 90.2 ± 1.5 % effect $p < 0.01$; day 5: 34.1 ± 9.7 % effect $p > 0.05$).

**MIA-induced osteoarthitic pain**. MIA injection into the knee joint induces osteoarthritis of the joint associated with weight bearing differences between the injured and non-injured hind limbs (Bove et al., 2003). Four days after MIA injection, substantial WBD was observed between the injured and non-injured hind limbs (48.1 ± 4.9 g, Figure 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, Figure 11).

**Spinal nerve injury-induced mechanical allodynia.** Spinal nerve injury (L5-L6 spinal nerve ligation. Chung model) induced a decrease in $PWT_{vonfrey}$ to mechanical stimulation with von Frey monofilaments 2 weeks following injury ($PWT_{vonfrey}$ control 13.7 ± 0.3 g, $PWT_{vonfrey}$ 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. ($PWT_{vonfrey}$ 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 $PWT_{vonfrey}$ to mechanical stimulation with von Frey monofilaments 2 weeks following surgery ($PWT_{vonfrey}$ control 11.3 ± 0.6 g, $PWT_{vonfrey}$ 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. ($PWT_{vonfrey}$ 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 naïve rats or naïve CD1 mice. In the rat, A-425619 (100 µmol/kg, i.p.) treated animals had a PWL to acute thermal stimulation of 8.6 ± 0.4 seconds compared to 8.2 ± 0.3 in vehicle-treated animals ($p > 0.05$, Table 1). In the mouse hot-plate assay, A-425619 (100 µmol/kg, i.p.) treated animals had a latency to jump of 81.6 ± 8.1 seconds compared to 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 100µmol/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 sec). A-425619 also had no significant effect on

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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 = $6882.4 \pm 493.9$). 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 post-operative pain. The TRPV1 receptor has been shown to be a molecular integrator of various pro-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), LTB4 (Ferreira et al., 2004), and 12-HPETE (Shin et al., 2002) are produced under inflammatory conditions. While 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 NGF 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 non-inflammatory thermal pain models. Thus, the *in vivo* behavioral effects of A-425619 are in close agreement with the phenotype observed in TRPV1 knock out 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 knock out as compared to wild-type mice (Caterina et al., 2000). Also in agreement with the TRPV1 knock out 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 knock out or by a selective receptor antagonist, is most effective in reducing inflammatory hyperalgesia.

Studies of TRPV1 receptor regulation have shown that TRPV1 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 towards 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 intrathecal 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 non-inflamed 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 non-injured 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 intrathecally delivered A-425619 to reduce sciatic nerve injury-induced mechanical allodynia. In addition, Gillen et al. (2004) have shown that intrathecal administration of TRPV1 antisense reduces mechanical allodynia observed after spinal nerve ligation and that intrathecal 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 post-operative 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-delta fibers and C fibers express the TRPV1 receptor (Michael and Priestley, 1999; Valtschanoff 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 non-injured 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 McCleane, 2000), this is the first demonstration of the antinociceptive effects of a selective TRPV1 receptor antagonist in an osteoarthritic 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 Valtschanoff (2004) showed increased TRPV1 expression in the peripheral axons of DRG neurons in the joint capsule of osteoarthitic 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 non-vanilloid TRPV1 receptor antagonists such as S*N*-(3-methoxyphenyl)-4-chlorocinnamide (SB-366791) (Gunthorpe et al., 2004), 4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (5-Trifluoromethyl pyridin -2-yl)amide (Compound 41) (Swanson et al. 2005), and ((*E*)-3-(4-*t*-butylphenyl)-

*N*-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)acrylamide (AMG9810) (Doherty et al., 2005; Gavva et al., 2005) have been identified in vitro as potent (15-100 nM at recombinant hTRPV1) TRPV1 receptor antagonists. Intraperitoneal administration of Compound 41 and AM9810 have been reported to block the algogenic effects of capsaicin in rats and AM9810 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, post-operative pain, and pain associated with osteroarthritis. The evaluation of A-425619 in these diverse pain models also significantly expands the antinociceptive profile for selective TRPV1 antagonists. These effects were observed in the absence of any locomotor impairment, and A-425619 maintained its antinociceptive 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 post-surgical pain.

## References

Appendino G, Harrison S, De Petrocelli L, Daddario N, Bianchi F, Schiano Moriello A, Trevisani M, Benvenuti F, Geppetti P and Di Marzo V (2003) Halogenation of a capsaicin analogue leads to novel vanilloid TRPV1 receptor antagonists. *Br J Pharmacol* 139(8):1417-1424.

Baron R, Neff L, Louvard D and Courtoy PJ (1985) Cell-mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-k D lysosomal membrane protein at the osteoclast ruffled border. *J Cell Biol* 101(6):2210-2222.

Bennett GJ and Xie YK (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33(1):87-107.

Blair HC, Teitelbaum SL, Ghiselli R and Gluck S (1989) Osteoclastic bone resorption by a polarized vacuolar proton pump. *Science* 245(4920):855-857.

Bove SE, Calcaterra SL, Brooker RM, Huber CM, Guzman RE, Juneau PL, Schrier DJ and Kilgore KS (2003) Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. *Osteoarth Cartil* 11(11):821-830.

Brennan TJ, Vandermeulen EP and Gebhart GF (1996) Characterization of a rat model of incisional pain. *Pain* 64(3):493-501.

Carlton SM and Coggeshall RE (2001) Peripheral capsaicin receptors increase in the inflamed rat hind paw: a possible mechanism for peripheral sensitization. *Neurosci Lett* 310(1):53-56.

Caterina MJ and Julius D (2001) The vanilloid receptor: a molecular gateway to the pain pathway. *Annu Rev Neurosci* 24:487-517.

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen ZKR, Koltzenburg M, Basbaum AI and Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288(5464):306-313.

Cho W and Valtschanoff JG (2004). Vanilloid receptor VR1 - positive primary afferents in mouse model of single joint inflammatory osteoarthritis. *Soc Neurosci Abstr*, San Diego.

Cortright DN and Szallasi A (2004) Biochemical pharmacology of the vanilloid receptor TRPV1 An update. *Eur J Biochem* 271(10):1814-1819.

Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A and Sheardown SA (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 405(6783):183-187.

Di Marzo V, Blumberg PM and Szallasi A (2002) Endovanilloid signaling in pain. *Curr Opin Neurobiol* 12(4):372-379.

Dinis P, Charrua A, Avelino A, Yaqoob M, Bevan S, Nagy I and Cruz F (2004) Anandamide-evoked activation of vanilloid receptor 1 contributes to the development of bladder hyperreflexia and nociceptive transmission to spinal dorsal horn neurons in cystitis. *J Neurosci* 24(50):11253-11263.

Doherty EM, Fotsch C, Bo Y, Chakrabarti PP, Chen N, Gavva N, Han N, Kelly MG, Kincaid J, Klionsky L, Liu Q, Ognyanov VI, Tamir R, Wang X, Zhu J,

Norman MH and Treanor JJS (2005) Discovery of potent, orally available vanilloid receptor-1 antagonists. structure-activity relationship of *N*-Aryl Cinnamides. *J Med Chem* 48:71-90.

El Kouhen R, Surowy CS, Bianchi BR, Neelands TR, McDonald HA, Niforatos W, Gomtsyan A, Lee C-H, Honore P, Sullivan JP, Jarvis MF and Faltynek CR (2005) A-425619, a novel and selective TRPV1 receptor antagonist, blocks channel activation by vanilloids, heat and acid. J Pharmacol Exp Ther (companion paper)

Ferreira J, da Silva GL and Calixto JB (2004) Contribution of vanilloid receptors to the overt nociception induced by B2 kinin receptor activation in mice. *Br J Pharmacol* 141:787-794.

Fukuoka T, Tokunaga A, Tachibana T, Dai Y, Yamanaka H and Noguchi K (2002) VR1, but not P2X3, increases in the spared L4 DRG in rats with L5 spinal nerve ligation. *Pain* 99(1-2):111-120.

Gavva NR, Tamir R, Qu Y, Klionsky L, Zhang TJ, Immke D, Wang J, Zhu D, Vanderah TW, Porreca F, Doherty EM, Norman MH, Wild KD, Bannon AW, Louis J-C and Treanor JJS (2005) AMG 9810 [(*E*)-3-(4-*t*-Butylphenyl)-*N*-(2,3-dihydrobenzo[*b*][1,4] dioxin-6-yl)acrylamide], a novel vanilloid receptor 1 (TRPV1) antagonist with antihyperalgesic properties. *J Pharmacol Exp Ther* 313:474-484.

Gillen C, Kurreck J, Christoph T, Grunweller A, Mika J, Schafer MKH, Weihe E, Schiene K and Erdmann VA (2004). Antinociceptive effect of antisense

oligonucleotides and siRNAs against VR1/TRPV1. *Soc Neurosci Abstr*, San Diego.

Gomtsyan A, Bayburt EK, Schmidt RG, Zheng G-Z, Perner RJ, Didomenico S, Koenig JR, Turner S, Jinkerson T, Drizin I, Hannick SM, Macri BS, McDonald HA, Honore P, Wismer CT, Marsh KC, Wetter J, Stewart KD, Oie T, Jarvis MF, Surowy CS, Faltynek CR, Lee C-H (2005) Novel transient receptor potential vanilloid 1 (TRPV1) receptor antagonists for the treatment of pain: structure–activity relationships for ureas with quinoline, isoquinoline, quinazoline, phthalazine, quinoxaline and cinnoline moieties. *J Med Chem* 48:744-752.

Gunthorpe MJ, Rami HK, Jerman JC, Smart D, Gill CH, Soffin EM, Hannan SL, Lappin SC, Egerton J, Smith JD, Worby A, Howett L, Owen D, Nasir S, Davies CH, Thompson M, Wyman PA, Randall AD and Davis JB (2004) Identification and characterisation of SB-366791, a potent and selective vanilloid receptor (VR1/TRPV1) antagonist. *Neuropharmacology* 46:133-149.

Haley JE, Sullivan AF and Dickenson AH (1990) Evidence for spinal N-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. *Brain Res* 518(1-2):218-226.

Hudson LJ, Bevan S, Wotherspoon G, Gentry C, Fox A and Winter J (2001) VR1 protein expression increases in undamaged DRG neurons after partial nerve injury. *Eur J Neurosci* 13(11):2105-2114.

Irie K, Hara I, Fumiko, Ozawa H and Yajima T (2002) Calcitonin gene-related peptide CGRP-containing nerve fibers in bone tissue and their involvement in bone remodeling. *Microsc Res Techni* 58(2):85-90.

Kamei J, Zushida K, Morita K, Sasaki M and Tanaka S (2001) Role of vanilloid VR1 receptor in thermal allodynia and hyperalgesia in diabetic mice. *Eur J Pharmacol* 422(1-3):83-86.

Kim SH and Chung JM (1992) An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain* 50(3):355-363.

Luo H, Cheng J, Han JS and Wan Y (2004) Change of vanilloid receptor 1 expression in dorsal root ganglion and spinal dorsal horn during inflammatory nociception induced by complete Freund's adjuvant in rats. *Neuroreport* 15(4):655-658.

Mach DB, Rogers SD, Sabino MC, Luger NM, Schwei MJ, Pomonis JD, Keyser CP, Clohisy DR, Adams DJ and Mantyh PW (2002) Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. *Neuroscience* 113(1):155-166.

McCleane G (2000) The analgesic efficacy of topical capsaicin is enhanced by glyceryl trinitrate in painful osteoarthritis: a randomized, double blind, placebo controlled study. *Euro J Pain* 4(4):355-360.

Michael GJ and Priestley JV (1999) Differential expression of the mRNA for the vanilloid receptor subtype 1 in cells of the adult rat dorsal root and nodose ganglia and its down-regulation by axotomy. *J Neurosci* 19(5):1844-1854.

Pogatzki EM, Gebhart GF and Brennan TJ (2002) Characterization of Adelta- and C-fibers innervating the plantar rat hind paw one day after an incision. *J Neurophysiol* 87(2):721-731.

Pomonis JD, Harrison JE, Mark L, Bristol DR, Valenzano KJ and Walker K (2003) N-4-Tertiarybutylphenyl-4-3-cholorphyridin-2-yltetrahydropyrazine-12H-carbox-amide BCTC, a novel, orally effective vanilloid receptor 1 antagonist with analgesic properties: II in vivo characterization in rat models of inflammatory and neuropathic pain. *J Pharmacol Exp Ther* 306:387-393.

Rashid M, Harunor, Inoue M, Bakoshi S and Ueda H (2003a) Increased expression of vanilloid receptor 1 on myelinated primary afferent neurons contributes to the antihyperalgesic effect of capsaicin cream in diabetic neuropathic pain in mice. *J Pharmacol Exp Ther* 306**:**709-717.

Rashid M, Harunor, Inoue M, Kondo S, Kawashima T, Bakoshi S and Ueda H (2003b) Novel expression of vanilloid receptor 1 on capsaicin-insensitive fibers accounts for the analgesic effect of capsaicin cream in neuropathic pain. *J Pharmacol Exp Ther* 304(3):940-948.

Shin J, Cho H, Hwang SW, Jung J, Shin CY, Lee S-Y, Kim SH, Lee MG, Choi YH, Kim J, Haber NA, Reichling DB, Khasar S, Levine JD and Oh U (2002) Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. *Proc Natl Acad Sci USA* 99:10150-10155.

Swanson DM, Dubin AE, Shah C, Nasser N, Chang L, Dax SL, Jetter M, Breitenbucher JG, Liu C, Mazur C, Lord B, Gonzales L, Hoey K, Rizzolio M, Bogenstaetter M, Codd EE, Lee DH, Zhang S-P, Chaplan SR and Carruthers NR (2005) Identification and Biological Evaluation of 4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (5-Trifluoromethylpyridin-2-yl)amide, a high

affinity TRPV1 (VR1) vanilloid receptor antagonist. *J Med Chem* 48(6):1857-1872.

Tohda C, Sasaki M, Konemura T, Sasamura T, Itoh M and Kuraishi Y (2001) Axonal transport of VR1 capsaicin receptor mRNA in primary afferents and its participation in inflammation-induced increase in capsaicin sensitivity. *J Neurochem* 76(6):1628-1635.

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI and Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 21(3):531-543.

Tominaga M, Wada M and Masu M (2001) Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. *Proc Natl Acad Sci USA* 98:6951-6956.

Valtschanoff JG, Rustioni A, Guo A and Hwang SJ (2001) Vanilloid receptor VR1 is both presynaptic and postsynaptic in the superficial laminae of the rat dorsal horn. *J Comp Neurol* 436(2):225-235.

Wahl P, Foged C, Tullin S and Thomsen C (2001) Iodo-resiniferatoxin, a new potent vanilloid receptor antagonist. *Mol pharmacol* 59(1):9-15.

Walker KM, Urban L, Medhurst SJ, Patel S, Panesar M, Fox AJ and McIntyre P (2003) The VR1 antagonist capsazepine reverses mechanical hyperalgesia in models of inflammatory and neuropathic pain. *J Pharmacol Exp Ther* 304(1):56-62.

Woo YC, Park S, Subieta AR and Brennan TJ (2004) Changes in tissue pH and temperature after incision indicate acidosis may contribute to postoperative pain. *Anesthesiology* 101(2):468-475.

**Legends for Figures**

**Figure 1.** Pharmacokinetic profile of A-425619 (10µmol/kg, i.v., i.p., and p.o.) in rats

**Figure 2.** Antinociceptive effects of A-425619 following intraplantar administration on capsaicin-induced mechanical hyperalgesia. In this experiment, A-425619 was administered 15 minutes before capsaicin injection and the animals tested 30 minutes after the injection of capsaicin. Data represent mean ± S.E.M. $F(3;28)=3.27$, $p < 0.02$. * $p < 0.05$, ** $p < 0.01$ as compared to vehicle-treated animals that received capsaicin into the paw. + $p < 0.05$, ++ $p < 0.01$ as compared to vehicle-treated animals that received vehicle into the paw. (n = 8 per group).

**Figure 3.** Antinociceptive effects of A-425619 following oral administration on capsaicin-induced mechanical hyperalgesia. In this experiment, A-425619 was administered 60 minutes before capsaicin injection and the animals tested 30 minutes after the injection of capsaicin. Data represent mean ± S.E.M. $F(5;42)=4.939$, $p < 0.002$. * $p < 0.05$, ** $p < 0.01$ as compared to vehicle-treated animals that received capsaicin into the paw. + $p < 0.05$, ++ $p < 0.01$ as compared to vehicle-treated animals that received vehicle into the paw. (n = 8 per group).

**Figure 4.** Antinociceptive effects of A-425619 following i.p. or p.o. administration in the CFA model of chronic inflammatory pain testing for thermal

hyperalgesia. Two days following CFA injection, A-425619 was injected 30 minutes (i.p.) or 60 minutes (p.o.) before testing. A-425619 demonstrated significant antihyperalgesic effects following both i.p. administration (A; $F(7;40)=18.374$, $p < 0.0001$) and oral administration (B; $F(7;86)=28.359$, $p < 0.0001$). Circles represent paw withdrawal latencies ipsilateral to the injury; Squares represent paw withdrawal latencies contralateral to the injury. Data represent mean ± S.E.M. * $p < 0.05$, ** $p < 0.01$ as compared to vehicle-treated animals. + $p < 0.05$, ++ $p < 0.01$ as compared to CFA-treated paw. (n = 6-12 per group).

**Figure 5.** Time course for the onset and duration of A-425619 (100 µmol/kg, i.p.) mediated anti-hyperalgesia (circles) in the rat. Squares represent paw withdrawal latencies of CFA-injected paws from animals treated with vehicle. Testing started 2 days following CFA injection and animals were tested 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, 480 minutes following dosing and one more time 24 hours following A-425619 injection. Data represent mean ± S.E.M. Repeated ANOVA $F(1;12)=9.594$, $p < 0.0001$. * $p < 0.05$, ** $p < 0.01$ as compared to vehicle-treated animals (n = 6 per group).

**Figure 6.** Antinociceptive effects of A-425619 following intraplantar or intrathecal administration in the CFA model of chronic inflammatory pain testing for thermal hyperalgesia. Two days following CFA injection, A-425619 was injected 30 minutes (intraplantar) or 5 minutes (intrathecal) before testing. A-425619 demonstrated significant antihyperalgesic effects following both

intraplantar administration (A; $F(9;50)=19.848$, $p < 0.0001$) and intrathecal administration (B; $F(7;40)=14.830$, $p < 0.0001$). Data represent mean ± S.E.M. ** $p < 0.01$ as compared to vehicle-treated animals. ## $p < 0.01$ between the effects of 300nmol injected in the injured paw or the non-injured paw (n = 6 per group).

**Figure 7.** Antinociceptive effects of A-425619 following i.p. administration in the carrageenan model of acute inflammatory pain testing for thermal hyperalgesia. Circles represent paw withdrawal latencies ipsilateral to the injury; Squares represent paw withdrawal latencies contralateral to the injury. Data represent mean ± S.E.M. $F(7;40)=38.065$, $p < 0.0001$. * $p < 0.05$, ** $p < 0.01$ as compared to vehicle-treated animals. + $p < 0.05$, ++ $p < 0.01$ as compared to carrageenan-treated paw. (n = 6 per group).

**Figure 8.** Antinociceptive effects of A-425619 in the skin incision model of post-operative pain, testing for thermal hyperalgesia 2 hours after surgery following i.p. (A; $F(7;40)=35.452$, $p < 0.0001$) and p.o. (B; $F(9;86)=53.635$, $p < 0.0001$) administration. In these experiments, following surgery, A-425619 was injected 30 minutes (i.p.) or 60 minutes (p.o.) before testing. Circles represent paw withdrawal latencies ipsilateral to the injury; Squares represent paw withdrawal latencies contralateral to the injury. Data represent mean ± S.E.M. * $p < 0.05$, ** $p < 0.01$ as compared to vehicle-treated animals. + $p < 0.05$, ++ $p < 0.01$ as compared to incised paw. (n = 6-12 per group).

**Figure 9.** Antinociceptive effects of A-425619 (i.p.) in the skin incision model of post-operative pain, testing for thermal hyperalgesia 24 hours ($F(7;40)=460.500$, $p < 0.0001$) after surgery. In this experiment, 24 hours post-surgery, A-425619 was injected i.p. 30 minutes before testing. Circles represent paw withdrawal latencies ipsilateral to the injury; Squares represent paw withdrawal latencies contralateral to the injury. Data represent mean ± S.E.M. * $p < 0.05$, ** $p < 0.01$ as compared to vehicle-treated animals. ++ $p < 0.01$ as compared to incised paw. (n = 6 per group).

**Figure 10.** Antinociceptive effects of A-425619 in skin-incision-induced post-operative pain following chronic dosing. In this experiment, vehicle (V) or A-425619 (A, p.o.) was injected 1 hour following surgery and animal tested for mechanical allodynia 2 hours following surgery. The same day (day 1), animals received a second injection of vehicle or A-425619 12 hours later. On day 2, 3, 4, and 5, animals received an injection of vehicle or A-425619 in the morning and were tested 60 minutes later for mechanical allodynia. On day 2, 3, and 4, animals received a second injection 12 hours later. Contra represents the withdrawal threshold for the contralateral side to the surgery. Data represent mean ± S.E.M. * $p < 0.05$, ** $p < 0.01$ as compared to vehicle-treated animals. (n = 6 per group).

**Figure 11.** Antinociceptive effects of A-425619 (i.p.) in osteoarthritic pain observed after MIA injection into the knee joint. Pain was evaluated by weight-

bearing difference, on day 4 post-MIA injection, 30 minutes following A-425619 administration. Circles represent weight bearing difference between injured and non-injured paw. Data represent mean ± S.E.M. $F(3;47)=15.2797$, $p < 0.0001$. * $p < 0.05$, ** $p < 0.01$ as compared to vehicle-treated animals. (n = 12 per group).

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## Tables

Table 1. Analgesic Profile of A-425619 (i.p.)

| **Pain Model** | **$ED_{50}$ µmol/kg i.p.** | **% Effect @ 100 µmol/kg i.p.** |
|---|---|---|
| **Acute Nociception** | | |
| Rat Acute Thermal | >100 | 3 ± 3% |
| Mouse Hotplate | >100 | 9 ± 1% |
| **Inflammatory Pain** | | |
| Formalin Test (Persistent phase) | >100 | 13 ± 4% |
| Acute Thermal Hyperalgesia<br>Edema<br>(Carrageenan) | 50<br>>100 | 78 ± 10%*<br>35 ± 17% |
| Chronic Thermal Hyperalgesia<br>(Complete Freund's Adjuvant) | 51 | 64 ± 15%* |
| **Neuropathic Pain** | | |
| Chronic Constriction Injury | >100 | 36 ± 4%* |
| L5/L6 Nerve Ligation | >100 | 36 ± 5%* |
| **Post-Operative Somatic Pain** | | |
| Acute Thermal (2 hours post-surgery) | 65 | 56 ± 8%* |
| Acute Mechanical (2 hours post-surgery) | >100 | 19 ± 6%* |
| Thermal (24 hours post-surgery) | >100 | 44 ± 3%* |
| Mechanical (24 hours post-surgery) | >100 | 42 ± 6%* |
| **Osteoarthritic Pain** | | |
| Weight Bearing Difference | >100 | 24 ± 10% |

* Significantly different ($p < 0.05$) from vehicle treated animals (n = 6-12 per group)

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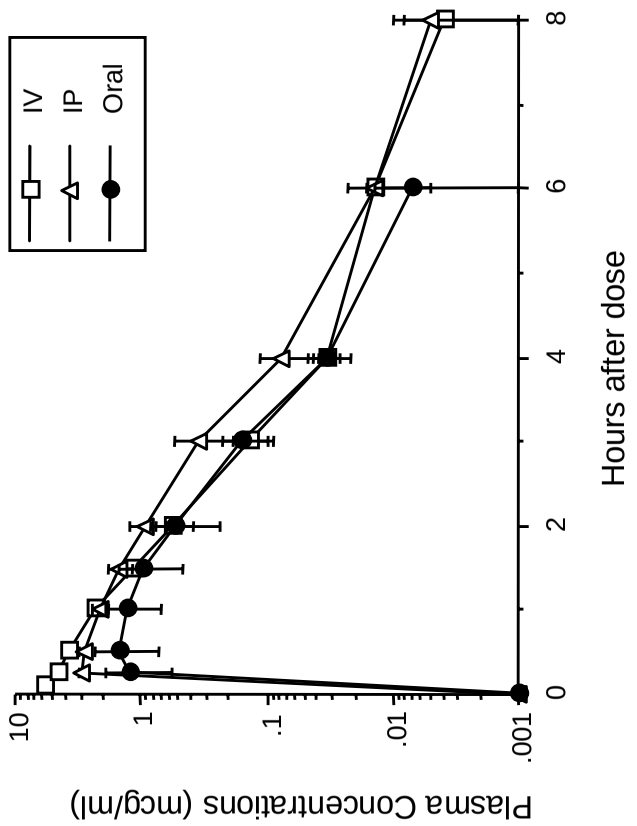


**Figure 1**

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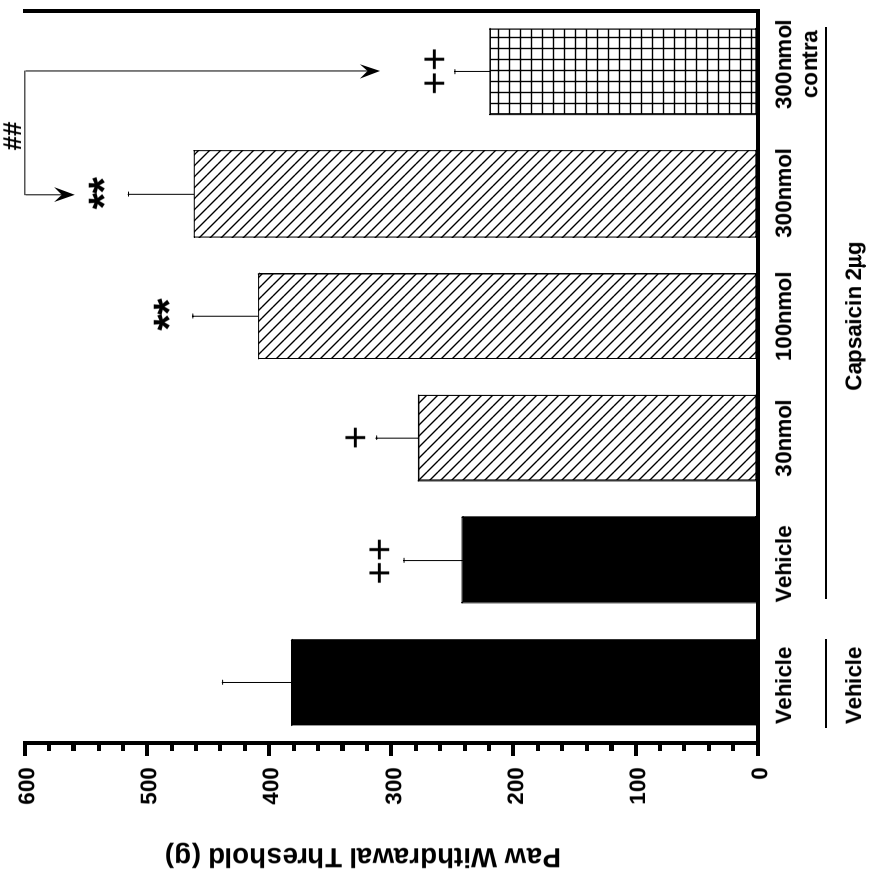


Figure 2

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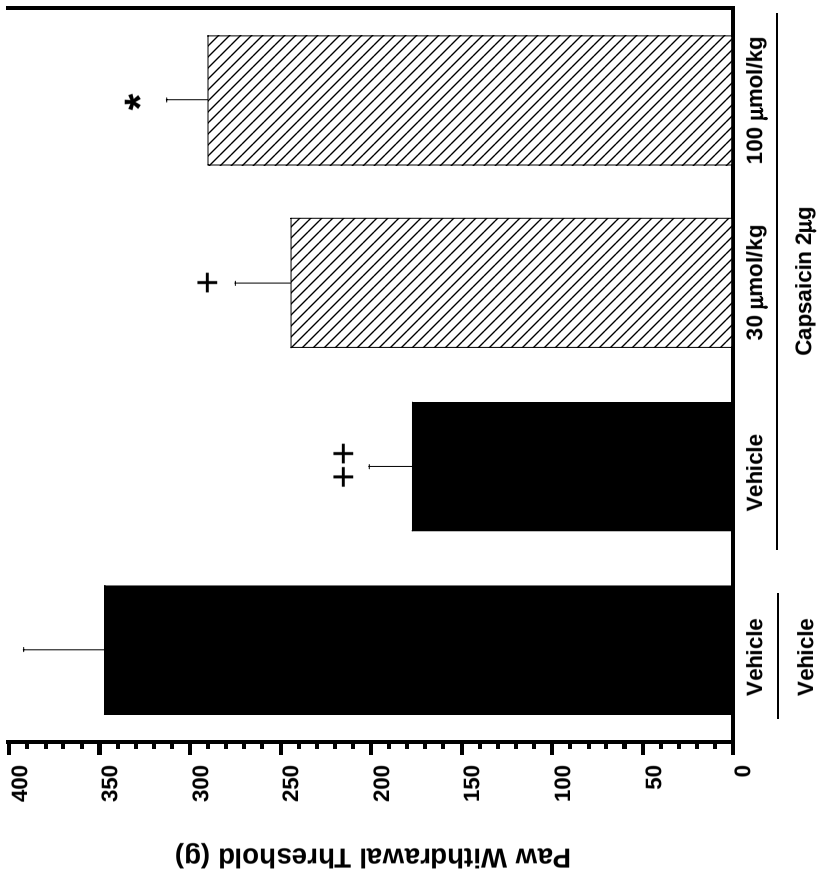
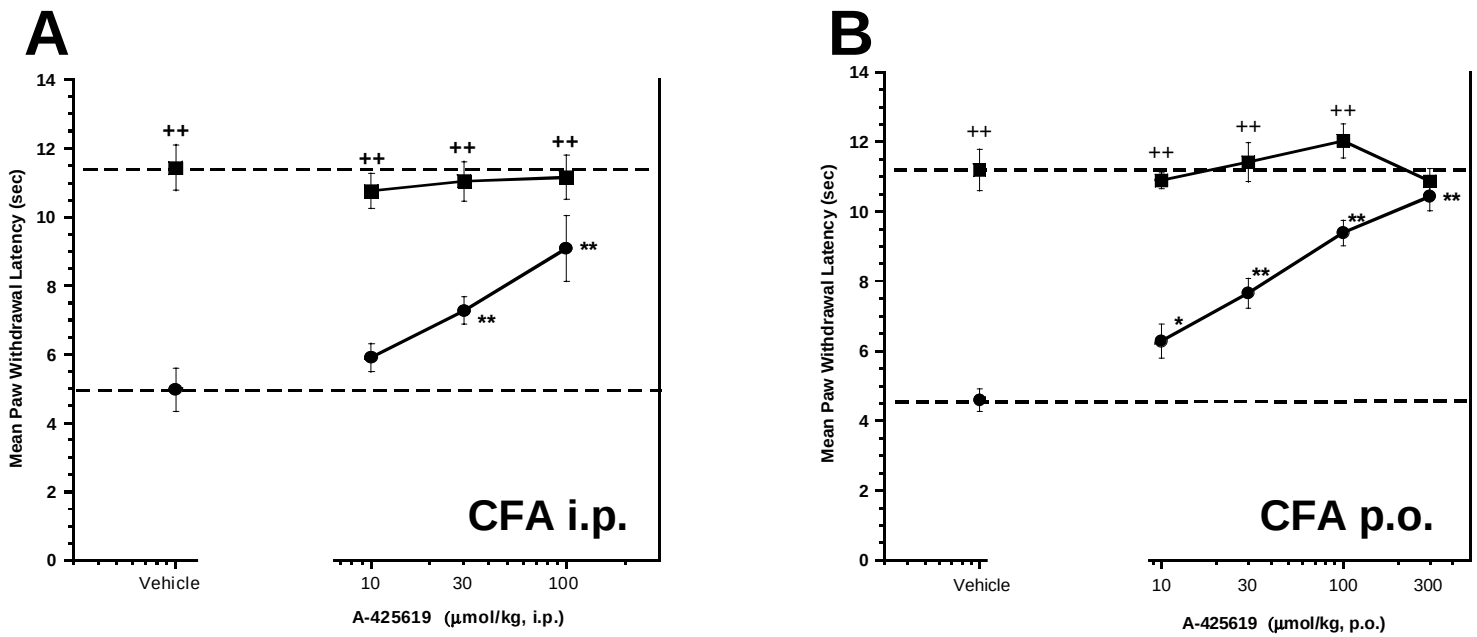



Figure 3

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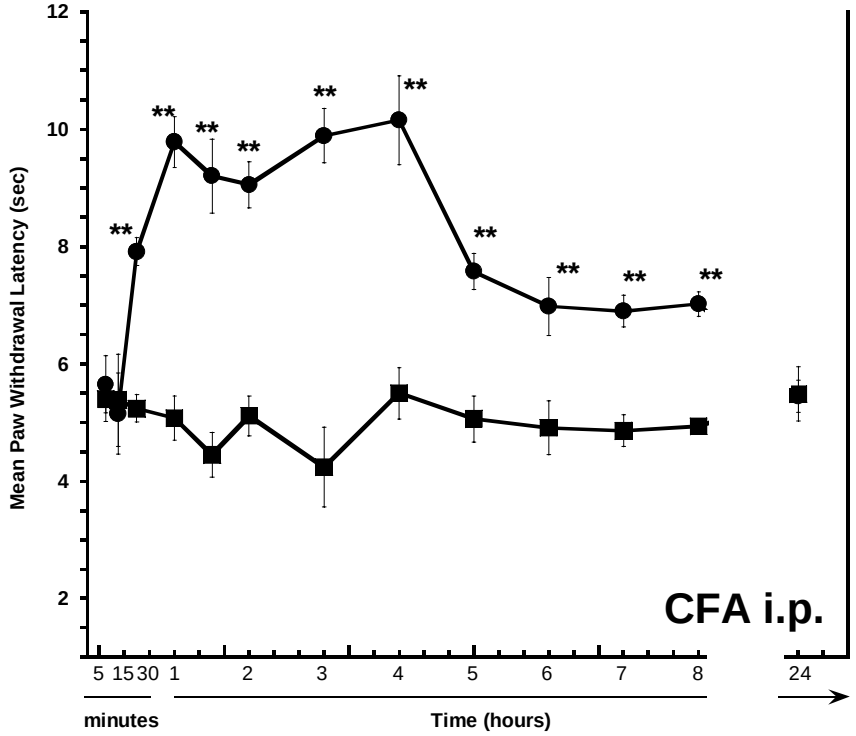


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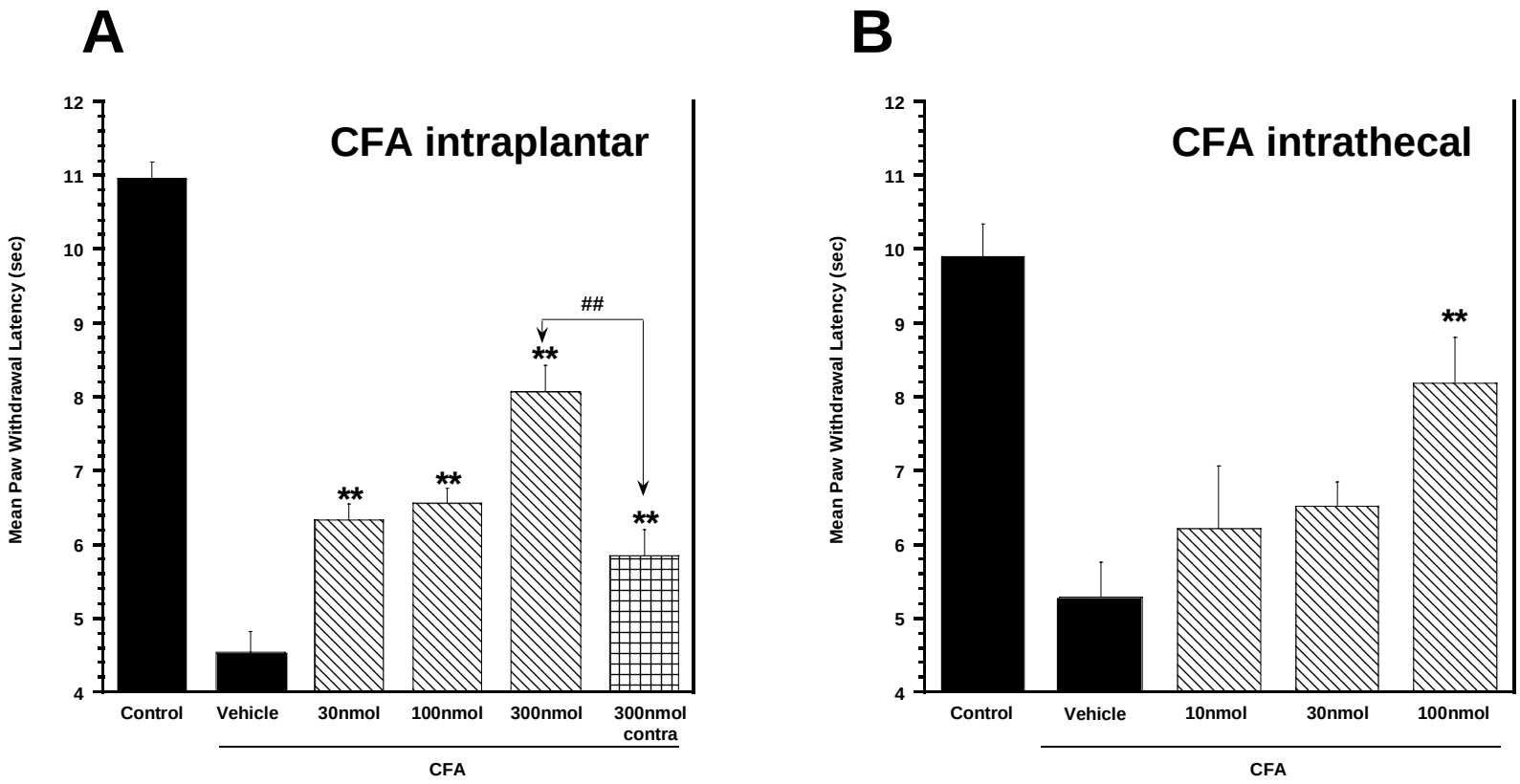
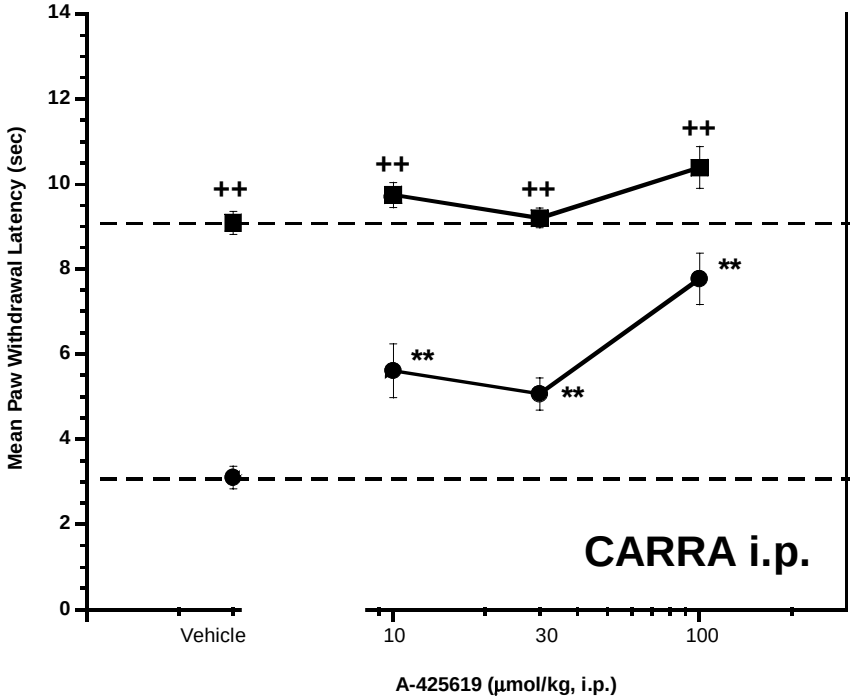



Figure 6

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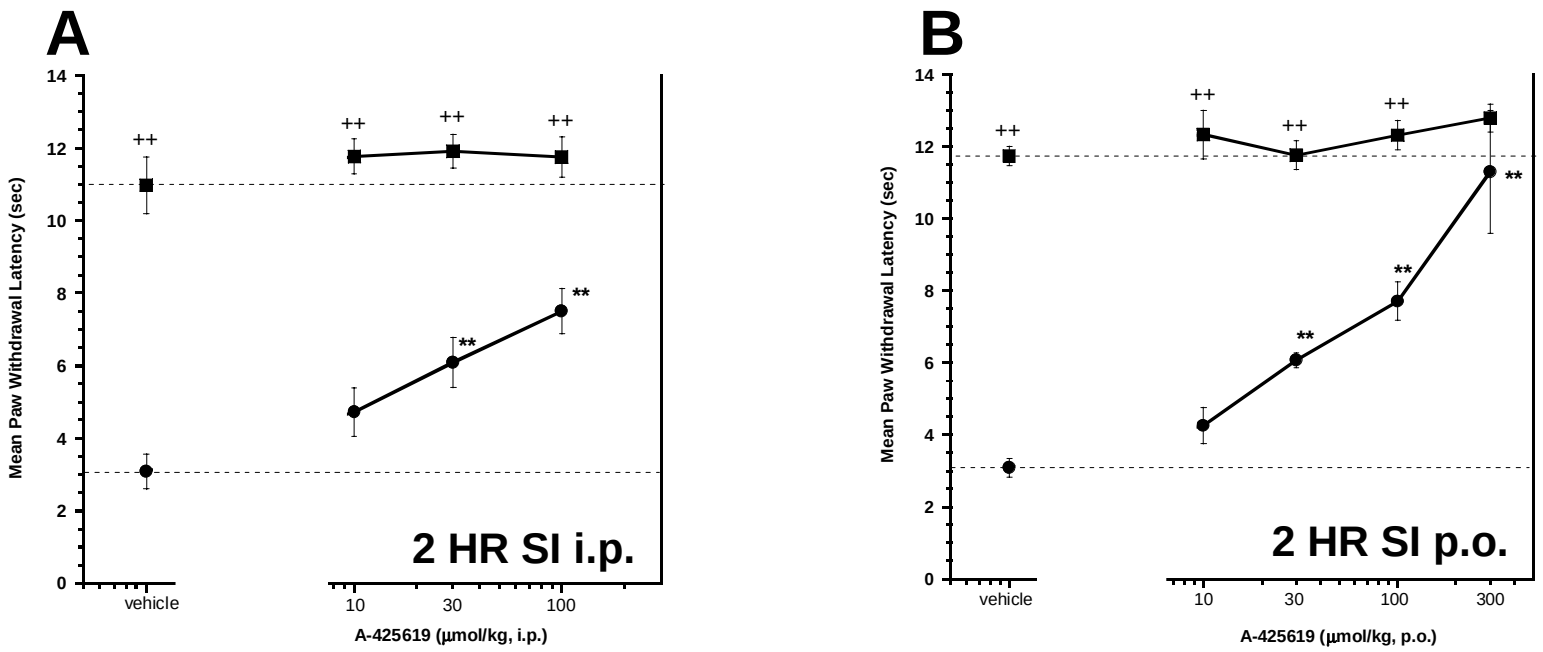


**Figure 8**

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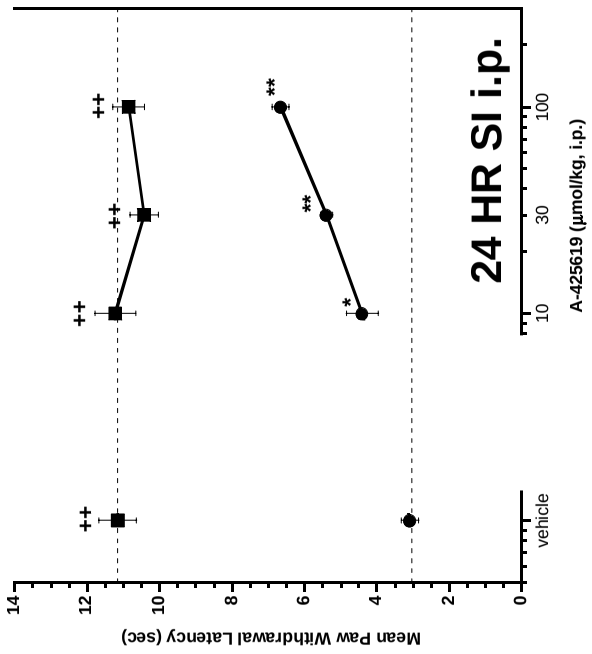


**Figure 9**

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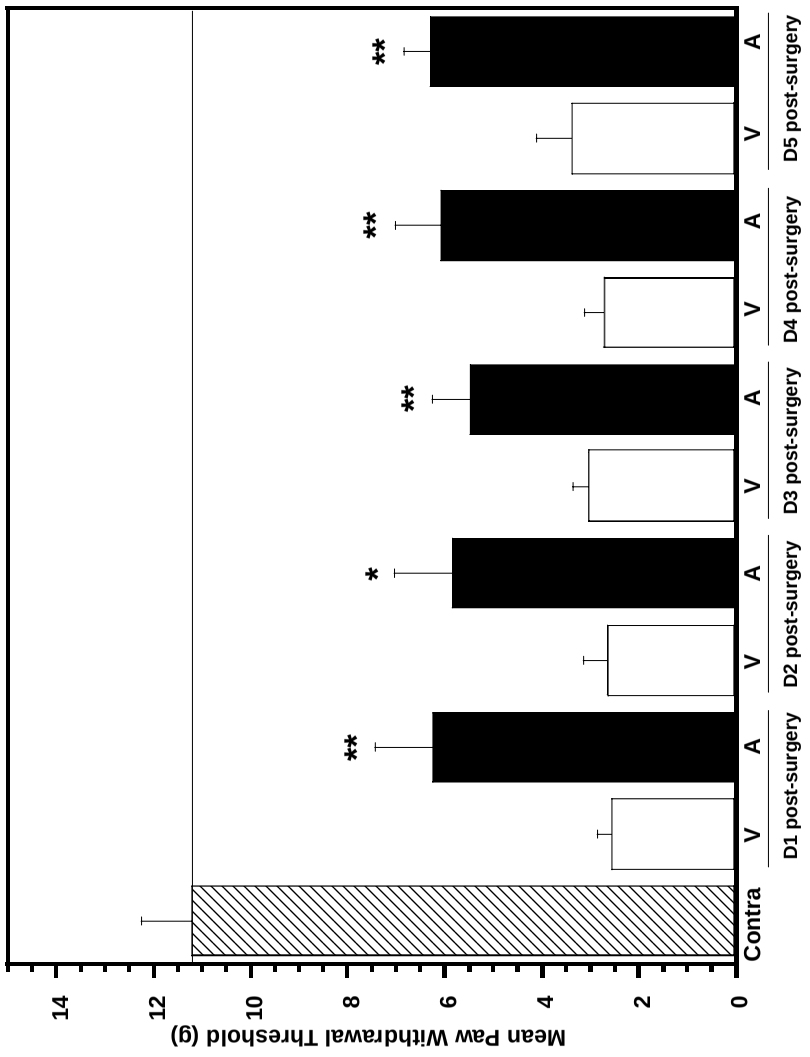


Figure 10

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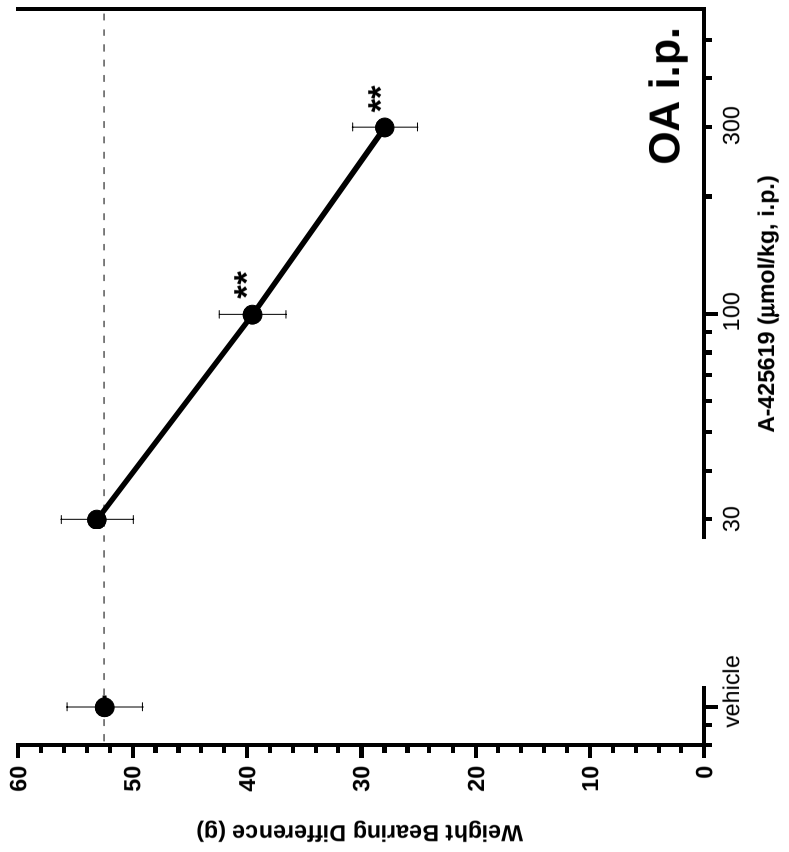


Figure 11