SYSTEMIC AND SITE-SPECIFIC EFFECTS OF A-425619, A SELECTIVE TRPV1 RECEPTOR ANTAGONIST, ON WIDE DYNAMIC RANGE NEURONS IN CFA-TREATED AND UNINJURED RATS

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ABSTRACT

Systemic administration of A-425619, a potent and selective TRPV1 receptor antagonist that does not readily enter the CNS, produces antinociception in several rat models of pathological nociception, including complete Freund’s adjuvant (CFA)-induced thermal hyperalgesia. In order to further understand the peripheral mechanisms of TRPV1-related antinociception, we examined the effects of systemic and site-specific injections of A-425169 on evoked and spontaneous firing of spinal wide dynamic range (WDR) neurons in uninjured rats and rats with peripheral inflammation (CFA, 48 hrs). In uninjured rats, capsaicin-evoked (1 µg) WDR activity was completely blocked by intraplantar administration of A-425619 (3-100 nmol). Systemic injection of A-425619 (3-30 µmol/kg, i.v.) reduced WDR responses to thermal stimulation in both CFA-inflamed (47°C) and uninjured (52°C) rats. However, the efficacy of A-425619 to attenuate thermal-evoked WDR activity was significantly greater (P < 0.01) in CFA-treated rats. Both intra-dorsal root ganglion (DRG, L5, 20 nmol) and intraplantar (30-300 nmol) injection of A-425619 reduced WDR responses to thermal stimulation. While the effectiveness of A-425619 was similar between CFA-inflamed and uninjured rats following intraplantar injection, the effects of A-425619 after intra-DRG injection were enhanced in the inflamed rats (compared to the uninjured rats). Spontaneous WDR discharges were unaltered by systemic or site-specific injections of A-425619. Thus, noxious thermal stimulation triggers the transmission of TRPV1-related signals to spinal WDR neurons in both inflamed and uninjured animals. The apparent increase in TRPV1 signalling to WDR neurons following injury may be the result of changes to the distribution/sensitization of peripheral TRPV1 receptors.
Key Words: TRPV1, WDR, A-425619, CFA, Hyperalgesia
INTRODUCTION

The vanilloid receptor, TRPV1, is a member of the transient receptor potential (TRP) family of ion channels that has a significant role in the transmission and modulation of nociceptive signals (DiMarzo et al., 2002). The TRPV1 receptor is found on small and medium sized primary afferent sensory neurons (Caterina et al., 1997; Guo et al., 1999; Ma, 2002; Tominaga et al., 1998), both presynaptically in lamina I and postsynaptically in lamina II of the spinal cord (Gou et al., 1999; Valschanoff et al., 2001), and in several supraspinal sites including regions that are implicated in nociceptive modulation (Acs et al., 1996; McGaraughty et al., 2003; Mezey et al., 2000; Szabo et al., 2002; Roberts et al., 2004). The primary afferent TRPV1 receptor is postulated to be a molecular integrator due to its heterologous activation and/or modulation by heat, protons and other endogenous matter released during tissue injury (Caterina et al., 1997; Chuang et al., 2001; Tominaga et al., 1998, 2001; Vellani et al., 2001; Vyklicky et al., 1998). Endogenous ligands such as anandamide, N-arachidonoyl-dopamine (NADA), and lipoxygenase derivatives may activate TRPV1 receptors located both inside and outside of the central nervous system (DiMarzo et al., 2002; Huang et al., 2002; Hwang et al., 2000; Sagar et al., 2004; Zygmunt et al., 1999).

The TRPV1 receptor is an important mediator of thermal nociception. Pharmacological block and/or inactivation of TRPV1 receptors attenuate responses to noxious heat stimulation in uninjured animals (Garcia-Martinez et al., 2002; Kelly and Chapman, 2002; McGaraughty, et al., 2003; Neubert et al., 2003). Furthermore, TRPV1 gene ablation in mice results in longer withdrawal latencies to intense thermal stimulation (Caterina et al., 2000). Following a chronic inflammatory or neuropathic injury, TRPV1
expression increases in the superficial layers of the spinal cord, and there is a
disproportionate upregulation of TRPV1 receptors on myelinated (compared to
unmyelinated) primary afferent neurons (Amaya et al., 2003; Hong and Wiley, 2005;
Hudson et al., 2001; Luo et al., 2004; Ma et al., 2005; Rashid et al., 2003). These
alterations in the distribution of TRPV1 receptors following injury likely reflect an
important contribution of these receptors to thermal hyperalgesia and allodynia (Caterina
et al., 2000; Kamei et al., 2001; Neubert et al., 2003; Rashid et al., 2003).

It has been recently reported that systemic administration of A-425619 (Figure 1),
a novel antagonist that has a high affinity and selectivity for blocking TRPV1 receptors,
is antinociceptive in several animal models of pathological nociception (El Kouhen et al.,
2005; Gomtsyan et al., 2005; Honore et al., 2005). A-425619 does not readily enter the
central nervous system when administered systemically, and was most effective to reduce
thermal hyperalgesia following a chronic inflammatory injury caused by intraplantar
administration of complete Fruend’s adjuvant (CFA, Honore et al., 2005). In order to
further understand the peripheral mechanisms of TRPV1-related antinociception, we
examined the effects of systemic A-425169 on evoked and spontaneous firing of spinal
wide dynamic range (WDR) neurons in uninjured and CFA-inflamed rats. Furthermore,
we investigated the relative contributions of TRPV1 receptors located on peripheral
terminals and the dorsal root ganglion (DRG) to the effects of A-425619.

MATERIALS AND METHODS

All animal handling and experimental protocols were approved by Abbott’s
Institutional Animal Care and Use Committee (IACUC), and were conducted in
accordance with the ethical principles for pain-related animal research of the American
Pain Society. Male Sprague-Dawley rats (Charles River, MA, 350-400 g) were used for all experiments and were housed in a temperature controlled room with a 12/12-hr day/night cycle. Food and water were available ad libitum.

In order to induce chronic inflammatory hyperalgesia, a 150 µl solution of 1:1 CFA and phosphate buffered saline was injected subcutaneously into the plantar region of the rat’s right hindpaw 48 hrs prior to testing. On the day of neuronal recording, CFA-inflamed and naïve animals were initially anesthetized with pentobarbital (50 mg/kg, i.p.). A catheter was placed into the left and/or right external jugular vein(s), and a laminectomy was performed to remove vertebral segments T12-L3. For animals receiving an intra-DRG injection of compound, the L5 DRG was exposed and a small membrane incision was made distal to the DRG. PE-5 catheters (external PE-10, Marsil Enterprises, CA) were inserted through the membrane opening up to the DRG. Animals were then secured in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) supported by clamps attached to the vertebral processes on either side of the exposure site. The exposed lumbar area was first enveloped by agar and then filled with mineral oil. A stable plane of anesthesia was maintained throughout the experiment by a continuous infusion of propofol at a rate of 8-12 mg/kg/hr (i.v.). Body temperature was kept at approximately 37°C by placing the animals on a circulating water blanket.

Platinum-iridium microelectrodes (Frederick Haer, Brunswick, ME) were used to record extracellular activity of WDR neurons located in the dorsal horn of the spinal cord. WDR neurons responded in a graded manner to both non-noxious (tap, brush) and noxious (pinch, heat) stimuli applied to the right hindpaw. Generally, WDR neurons (Maixner et al., 1986; Price and Browe, 1975; Surmeier et al., 1986) have graded
responses to increasingly intense thermal stimulation and this was tested (described below) using the propofol protocol outlined above with temperatures ranging from 32-52°C. Spike waveforms were monitored on an oscilloscope throughout the experiment, digitized (32 points), and then stored for off-line analysis (SciWorks, Datawave Technologies, Longmont, CO) to ensure that the unit under study was unambiguously discriminated throughout the experiment. Except for 5 experiments in which two easily distinguished neurons were simultaneously recorded on one electrode, only one cell was studied in each experiment.

At the onset of each experiment, spontaneous neuronal firing was recorded for 5 min to determine baseline levels. Neurons were then characterized by their responses to innocuous (tap, brush, air puff) and noxious stimuli (pinch, heat) applied to the ipsilateral hindpaw. The hindpaw receptive field to pinch stimulation was subsequently mapped for each neuron. The actions of A-425619 on WDR neuronal activity were then examined under two different experimental paradigms. In the first paradigm, the effects of A-425619 on spontaneous and thermal-evoked activity were measured in uninjured and CFA-inflamed animals. The thermal stimulus was a glass reservoir filled with non-circulating water. The reservoir was the inner section of a double-walled glass-tempering beaker. The temperature of the stimulus was maintained by water circulating through the enclosed external chamber and was feedback regulated by a flow-through heater (Polyscience, Niles, IL). In order to produce similar levels of baseline evoked firing, uninjured and CFA-treated animals received different intensities of thermal stimulation. Since neuronal sensitization and behavioral hyperalgesia are typically observed from CFA-treated animals, these rats were presented with a lower temperature stimulus. Thus,
the ipsilateral hindpaw in uninjured rats was immersed in 52°C water for 10 s while the hindpaw of CFA-injected rats was immersed in 47°C water for 10 s. As a measure of baseline (pre-drug) evoked firing, the ipsilateral hindpaw for both groups was immersed in water for 10 s over three trials, each separated by 5 min. A-425619 or vehicle was then administered systemically (3-30 µmol/kg, i.v.), intraplantarly (30-300 nmol in 50 µl), or directly onto the L5 DRG (20 nmol in 2 µl). Thermal-evoked and spontaneous activity was then measured 1 (for DRG injections), 5, 15, and 25 min after A-425619 or vehicle injection.

To determine if the DRG injection technique resulted in site-specific drug effects on WDR neuronal activity, lidocaine (5% in saline) was administered either onto the L5 DRG (2 µl) using the technique described above or directly into spinal tissue (0.2 µl) using a technique described previously (Heinricher and McGaraughty, 1998). Briefly, for the latter protocol, a glass infusion pipette (outer diameter 75-80 µm) with an angled beveled tip was attached to the recording electrode in such a way that the tips were separated by about 300 µm laterally and by 30-100 µm dorsoventrally. The electrode and pipette were simultaneous lowered into the spinal tissue. The infusion pipette was attached to a 1 µl Hamilton syringe with a length of PE-50 tubing for drug infusion.

The second paradigm examined the effects of A-425619 to reduce capsaicin-evoked activity. In naïve animals, 10 µl of vehicle was injected into the neuronal receptive field to measure evoked firing caused by needle insertion and fluid injection. Two minutes later, A-425619 or vehicle was injected systemically (30 µmol/kg, i.v.) or intraplantarly (3-100 nmol in 50 µl). Capsaicin (1 µg in 10 µl) was then administered into the receptive field 15 min after compound injection. Neuronal activity was recorded for
another 5 min after capsaicin administration. To determine capsaicin-evoked activity for each animal, neuronal discharges (1 min) caused by the injection of 10 µl of vehicle into the hindpaw were subtracted out of the neuronal activity caused by the hindpaw injection of 10 µl of capsaicin (1 min).

**Delivery and preparation of compounds**

A-425619 was synthesized at Abbott Laboratories (Abbott Park, IL) and was dissolved in 10% EtOH, 10% polyoxyethylene sorbitan monooleate, and saline for all injection routes. The vehicle for capsaicin (Sigma-Aldrich, St. Louis, MO) was 20% EtOH, 10% polyoxyethylene sorbitan monooleate and saline. For systemic injection, the solution was infused over a 5 to 7 min period at a volume of 1 ml/kg (1.2 - 12.11 mg/ml). Intraplantar injections (50 µl) were made to both the ipsilateral and contralateral hindpaws in separate experiments. For direct DRG injections, A-425619 (20 nmol in 2 µl) was injected over a period of 1 min onto the L5 DRG through the indwelling catheter attached to a 10 µl Hamilton syringe. If the intra-DRG injection of A-425619 or vehicle was without effect on neuronal activity, 5% lidocaine (1 µl) was then infused onto the L5 DRG. If evoked activity was unaffected by lidocaine, it was determined that the recorded spinal neuron did not receive direct/indirect input from the L5 DRG and was not used for data analysis.

**Data analysis**

For each rat, the post-drug spontaneous and thermal-evoked activity were calculated as a percent of their respective baseline levels. All data are presented as mean ± SEM. For comparisons to baseline firing levels, statistical significance was established by using a Wilcoxon’s matched-pairs test. A Kruskal-Wallis analysis of variance
followed by a Mann-Whitney U test was used for comparison across groups (P < 0.05). Except in one experiment, ED$_{50}$ values were calculated with respect to baseline activity and were estimated (eED$_{50}$) using linear regression. Since needle insertion and intraplantar injection of vehicle to CFA-inflamed rats caused significant increases in spontaneous and evoked firing from baseline levels, the eED$_{50}$ value in this case was calculated with respect to vehicle activity.

**RESULTS**

**Baseline neuronal activity**

Discharge activity was recorded from 152 WDR neurons and the mean depth was 788.1 ± 20.8 µm from the surface of the spinal cord. Baseline (pre-drug) levels of WDR spontaneous firing were significantly greater (P < 0.05) in CFA-inflamed than uninjured rats (Table 1), which is an indication of neuronal sensitization (Chu et al, 2004). As with other paradigms (Maixner et al., 1986; Price and Browe, 1975; Surmeier et al., 1986), WDR neurons recorded from rats anesthetized with the current propofol protocol (both CFA-inflamed and uninjured animals) responded incrementally to increasing intense thermal stimulation (Figure 2). In order to achieve similar levels of evoked WDR firing between the CFA-treated and naïve rats, stimulus intensity was adjusted to account for the hyperalgesic state of CFA-inflamed rats. To this end, hindpaws in CFA-treated animals were immersed in 47°C water while the hindpaws in the uninjured rats were immersed in 52°C. This adjustment did result in producing similar levels of evoked firing in the CFA-treated and uninjured rats (Table 1).
**Effects of A-425619 on thermal-evoked activity**

_Systemic administration._ Systemic administration (i.v.) of A-425619 (3-30 µmol/kg) dose-dependently attenuated thermal-evoked WDR activity in CFA-treated rats with an eED₅₀ of approximately 10 µmol/kg (Figure 3A). The systemic effect occurred within 5 min of injection and lasted for the duration of the recording period (Figure 2B). The effect of A-425619 (30 µmol/kg, i.v.) to attenuate evoked WDR firing was significantly greater (p < 0.01) in CFA-treated rats (66.1 ± 6.2% reduction) than in uninjured rats (18.6 ± 5.4% reduction; Figure 4). These effects of systemic A-425619 were not related to an effect on blood pressure since the achieved plasma concentrations (approximately 14.1 µg/ml) of the highest dose tested, 30 µmol/kg (i.v.), are well below the concentrations (76.8 µg/ml) needed to significantly reduce mean arterial pressure in anesthetized rats (J.A. Segreti and J.S. Polakowski, unpublished observation). In order to determine the contribution of specific sites to this difference in A-425619 efficacy, A-425619 was administered into the plantar region of the hindpaw and onto the L5 DRG in CFA-treated and uninjured rats.

_Intraplantar administration._ Needle insertion and administration of the 50 µl vehicle into the animal’s hindpaw significantly increased thermal-evoked WDR activity in CFA-inflamed but not uninjured animals in the first 5 min after injection. At 5 min after vehicle injection, evoked WDR activity in CFA-inflamed animals rose by 202.6 ± 69.7% compared to baseline (P < 0.01). By the next stimulus presentation at 15 min post-vehicle injection, the mean evoked response to thermal stimulation was still slightly elevated but was not significantly different than baseline levels. Similar effects on evoked activity in CFA-inflamed rats have been observed with an intraplantar injection of a
100% saline vehicle (McGaraughty and Chu, unpublished observations), suggesting that this was due to needle insertion and injection of a 50 µl volume into the neuronal receptive field of a sensitized paw, and not due to the 10% EtOH-based vehicle used in the current experiment.

Intraplantar administration of 100 and 300 nmol of A-425619 significantly (P < 0.05) reduced thermal-evoked WDR activity in both CFA-inflamed and uninjured rats (Figure 5). The efficacy of intraplantar A-425619 was similar between these two groups as the eED50's, 15 min after injection were 75 nmol (uninjured rats) and 85 nmol (CFA-treated rats). The significant anti-hyperalgesic effect of A-425619 lasted for the duration of the recording period (25 min post-injection) in both CFA-inflamed and uninjured rats (Figure 5B). Administration of A-425619 (100 and 300 nmol) into the contralateral hindpaw did not significantly alter WDR neuronal activity (data not shown).

Intra-DRG administration of A-425619. Injection of 20 nmol of A-425619 onto the L5 DRG significantly (P < 0.05) decreased WDR responses to thermal stimulation in both CFA-inflamed and uninjured rats. This effect was significantly (P < 0.05) greater in CFA-treated rats (Figure 6). The significant anti-hyperalgesic effect of intra-DRG A-425619 occurred within 1 min of injection and lasted for the entire 25 min of recording for both CFA–inflamed (Figure 6B) and uninjured rats.

Intra-DRG or intra-spinal administration of lidocaine. Possible diffusion of drug from the DRG to relevant spinal tissue using the current intra-DRG technique was investigated by comparing the effects of lidocaine on WDR neuronal activity following intra-DRG or intra-spinal injection. In both CFA and uninjured animals (data are combined), injection of 5% lidocaine onto the L5 DRG (in 2.0 µl) or directly into spinal
tissue (in 0.2 µl) significantly reduced thermal-evoked WDR activity 5 min after injection (Figure 7). However, the intra-spinal administration of lidocaine was significantly (P < 0.01) more effective to reduce WDR responses to thermal stimulation. In contrast, the spontaneous firing of WDR neurons was almost completely eliminated following the spinal injection of lidocaine, but was not significantly affected by the intra-DRG injection (Figure 7). This latter finding showing a lack of significant effects on spontaneous firing following the intra-DRG injection, suggests that using the present technique to administer compounds (in a volume of 2 µl) onto the L5 DRG does not result in significant diffusion of compound into spinal tissue to affect WDR neuronal firing.

**Effects of A-425619 on Spontaneous Activity**

Despite clear effects on evoked activity, systemic injection of A-425619 did not alter the spontaneous firing of WDR neurons in CFA-inflamed and uninjured rats (Figure 8). Spontaneous WDR firing was also unaltered by intraplantar or intra-DRG injection of A-425619 (data not shown).

**Effects of A-425619 on Capsaicin-evoked activity**

The effect of A-425619 on capsaicin-evoked activity was examined in order to: 1) evaluate A-425619’s efficacy against a specific TRPV1 mediated injury; and 2) to compare the intraplantar and systemic effects of A-425619 at doses used to attenuate thermal-evoked activity in uninjured animals. The number of capsaicin-evoked WDR discharges did not differ between groups given systemic or intraplantar administration of vehicle; therefore, the vehicle data from the two groups were combined. The injection of capsaicin into the neuronal receptive field of vehicle-treated rats evoked a total of 328.9 ± 58.8 WDR spikes in the first minute after injection. The WDR response to capsaicin was
completely blocked by pre-treatment with intraplantar A-425619 (100 nmol, Figure 9). Furthermore, the eED$_{50}$ (3-100 nmol) to attenuate capsaicin-evoked WDR firing following intraplantar injection of A-425619 was approximately 40 nmol (data not shown). Using the highest dose administered systemically to affect thermal-evoked activity in uninjured rats, pre-treatment with 30 µmol/kg (i.v.) of A-425619 reduced capsaicin-evoked activity by only 56% (145 ± 47.7 spikes, P< 0.05) compared to the vehicle group (Figure 9).

**DISCUSSION**

It has been recently reported that A-425619, a selective and potent TRPV1 receptor antagonist, is efficacious in a variety of animal models of pathophysiological nociception including CFA-induced hyperalgesia and its effects in this model last for at least eight hours after injection (El Kouhen et al., 2005; Honore et al., 2005). The antinociceptive actions of A-425619 were likely mediated by peripheral sites since A-425619 does not readily enter the CNS following systemic administration (Honore et al., 2005). The current data showing that systemic administration of A-425619 attenuated thermal-evoked activity of spinal WDR neurons in CFA-inflamed rats is consistent with the compound’s anti-hyperalgesic action in behavioral tests. Injection of A-425619 also reduced thermal-evoked discharges of WDR neurons in uninjured rats. However, the efficacy of systemic A-425619 to attenuate WDR activity was much greater in the inflamed rats, a difference also noted in behavioral tests, and suggests an increased role for TRPV1 receptors following an inflammatory injury.

The apparent increase in TRPV1-related utilization of WDR neurons following injury may be the result of changes to the distribution of TRPV1 receptors. Under normal
physiological conditions, TRPV1 receptors are localized to C- and A\(\delta\)-primary afferent fibers, and the distribution is weighted towards C-fibers (Caterina et al., 2000). Following a chronic injury, including CFA-induced inflammation, expression of TRPV1 receptors has been shown to increase to a much greater degree in the myelinated A\(\delta\)- and A\(\beta\)-fibers than in the unmyelinated C-fibers (Amaya et al., 2003; Hong and Wiley, 2005; Hudson et al., 2001; Luo et al., 2004; Ma et al., 2005; Rashid et al., 2003). This shift in distribution to the myelinated fibers could change the dynamics of TRPV1-related input to the spinal cord, and give heat, endovanilloids and TRPV1 sensitizing agents an additional or alternate means to affect neuronal activity in the spinal dorsal horn following injury. The present data suggest that A-425619 blocked this additional input from reaching WDR neurons in the inflamed rats. Although there is some evidence that A\(\beta\)-fibers have a “phenotype switch” following an inflammatory injury that affects spinal hypersensitivity (Neumann et al., 1996), and that these large diameter afferents are sensitive to temperature change following injury (Li et al., 2002), the redistribution of TRPV1 to A\(\delta\)-fibers was likely the important difference that resulted in the observed effects of A-425619 on thermal-evoked WDR activity (Djouhri and Lawson, 1999; 2004; Levine and Taiwo, 1994).

There are a couple of technical factors that must be considered when interpreting the present data. One of these factors is the difference in intensity of thermal stimulation presented to the CFA-treated (47°C) and uninjured (52°C) animals. In agreement with previous reports (Maixner et al., 1986; Price and Browe, 1975; Surmeier et al., 1986), it was demonstrated that WDR neurons from propofol-anesthetized rats have graded responses to increasingly intense thermal stimulation, and therefore a 47°C stimulus
evokes less neuronal activity than a 52°C stimulus. The intent of the current experimental paradigm was to produce a similar degree of baseline evoked neuronal firing in “sensitized” (CFA) and “normal” (uninjured) conditions. To achieve this, the CFA-inflamed animals were presented with a lower temperature stimulus. This stimulus adjustment was successful and the effects of A-425619 on WDR activity were measured against similar levels of baseline-evoked firing between inflamed and uninjured rats. Another factor to consider is the use of an ethanol-based vehicle. Due to the limited solubility of A-425619, addition of 10% ethanol was needed to dissolve the compound into a solution. However, an *in vitro* study has shown that ethanol potentiates TRPV1 responses to capsaicin and heat (Trevisani et al., 2002), and thus, may interfere with the degree of A-425619 efficacy observed in the current study.

In agreement with previous studies using chronically injured animals, CFA-induced inflammation altered the levels of WDR baseline activity (Chu et al., 2004; Hylden et al., 1989; Ren et al., 1992). To this end, WDR neurons in CFA-treated animals displayed significantly higher rates of spontaneous firing than the uninjured rats, and despite receiving a less intense stimulus, the degree of evoked WDR activity in CFA-inflamed rats was equivalent to the uninjured rats. However, administration of A-425619 affected evoked, but not spontaneous WDR firing. Heightened levels of spontaneous WDR firing that accompany a chronic injury are considered to be a result of central sensitization (Chapman et al., 1998; Chu et al., 2004; Pertovaara, et al., 2001; Sotgiu and Biella, 2000). Thus, the complete lack of effect on spontaneous WDR firing suggests that systemically administered A-425619 does not reduce central sensitization. Since the compound is reported to have poor CNS penetration (Honore et al., 2005), the most likely
explanation for this result is that insufficient levels of A-425619 entered the CNS to affect the elevated spontaneous firing of WDR neurons. Systemic administration of A-425619 also did not attenuate the nocifensive behaviors during the persistent (2\textsuperscript{nd}) phase of the formalin assay (Honore et al., 2005), an outcome which is consistent with a lack of effect on central sensitization (Coderre et al., 1990; Dickenson and Sullivan, 1987). Even though the antinociceptive effects of systemically administered A-425619 are probably mediated though peripheral sites of action, there is mounting evidence that central TRPV1 receptors modulate nociceptive activity and may even broaden the therapeutic effectiveness of a centrally acting TRPV1 agent (Doly et al., 2004; Honore et al., 2005; Kanai et al., 2005; Kelly and Chapman, 2002; Luo et al., 2004; McGaraughty et al., 2003; Palazzo et al., 2002).

TRPV1 receptors located on peripheral terminals likely contributed to the systemic action of A-425619, since intraplantar administration of A-425619 reduced the thermal- and capsaicin-evoked firing of WDR neurons. The effect of intraplantar A-425619 on thermal-evoked WDR neuronal activity was similar between CFA-treated and uninjured rats. This result contrasts with the differential effect of systemic A-425619 on thermal-evoked WDR firing between these groups of animals. Thus, the increased efficacy of systemically delivered A-425619 on WDR activity in inflamed rats cannot be accounted for by the compound’s action at the peripheral terminals. The increased efficacy may instead be related to the action of A-425619 on TRRV1 receptors located within the DRG. Direct injection of A-425619 onto the L5 DRG, likely affecting TRPV1 receptors in local cell bodies and axons, decreased thermal-evoked WDR activity to a greater degree in CFA-treated rats than in uninjured animals, a differential effect which
parallels the actions of systemic A-425619. This latter outcome is also consistent with the upregulation and re-distribution of TRPV1 receptors in the DRG following a chronic injury (Amaya et al., 2003; Hong and Wiley, 2005; Luo et al., 2004; Ma et al., 2005) and suggests that A-425619 reached functional TRPV1 receptors in the DRG following systemic delivery through the sites ample blood supply.

Nonetheless, a recent report by Jhaveri et al. (2005) has shown that intraplantar injection of another TRPV1 antagonist, iodo-resiniferatoxin, was more potent to reduce thermal-evoked (45°C) responses of WDR neurons in rats with carrageenan-induced acute inflammation than in uninjured rats. This is an outcome that is consistent with an injury-related upregulation of TRPV1 on peripheral terminals (Carlton and Coggeshall, 2001). A-425619 and iodo-resiniferatoxin are both selective antagonists of the TRPV1 receptor, but A-425619 (IC$_{50}$ = 5 nM) is 15-fold more potent than iodo-resiniferatoxin (IC$_{50}$ = 75 nM) in blocking capsaicin activation of the TRPV1 receptor (El Kouhen et al., 2005). It is not clear why the upregulation of TRPV1 receptors on peripheral terminals was not manifested by the intraplantar actions of A-425619. However, the differences between the effects of A-425619 and iodo-resiniferatoxin at the peripheral terminals may be related to distinctive physiochemical properties of the two compounds, or to differences in the stimulation protocols.

In the uninjured rats, systemic administration of A-425619 was not very effective to reduce thermal-related inputs to WDR neurons. Thus, the high degree of efficacy following intraplantar injection of A-425169 to the uninjured rats was somewhat unexpected. This disparity in A-425619 efficacy in the uninjured rats was likely a consequence of differences in achieved local concentrations of A-425619. Thus, the
levels of A-425619 at the peripheral terminals following systemic injection, at the highest
dose tested (30 µmol/kg), were probably much lower than those achieved following
intraplantar administration (at 100 nmol). Consistent with this hypothesis, while
intraplantar administration of A-425619 (100 nmol) completely blocked capsaicin-
evoked WDR activity, systemic delivery of A-425169 (30 µmol/kg) produced only about
a 56% attenuation in the capsaicin-related firing.

In summary, administration of a selective TRPV1 receptor antagonist, A-425619,
reduced thermal-evoked inputs to spinal WDR neurons. In agreement with behavioral
studies (Honore et al., 2005), the effect of systemic A-425619 increased following a
chronic inflammatory injury, which was manifested by a greater attenuation in the
evoked firing of WDR neurons. Injury-induced redistribution of TRPV1 receptors in the
DRG region is likely a significant factor leading to the increased effectiveness of
systemically delivered A-425619 in CFA-inflamed animals.
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Table 1. Baseline levels of evoked and spontaneous firing

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<th>Uninjured</th>
<th>CFA</th>
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<td>Spontaneous (spikes/s)</td>
<td>2.1 ± 0.4</td>
<td>4.1 ± 0.4**</td>
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<tr>
<td>Thermal-evoked (spikes/15 s)</td>
<td>357.1 ± 43.2 (52°C)</td>
<td>335 ± 23.1 (47°C)</td>
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**p< 0.01 vs. uninjured group
Figure 1. Structure of A-425619

Figure 2. WDR responses to increasing stimulus temperature in CFA and uninjured rats anesthetized with a continuous infusion of propofol. Shaded region on the paw represents the neuronal receptive field.

Figure 3. (A) Administration of systemic A-425619 dose-dependently (3-30 µmol/kg, i.v.) reduced thermal-evoked WDR activity in CFA-treated rats (n = 6-8 per group). (B) Representative ratemeter showing the thermal-evoked activity of a single WDR neuron both before (base) and after injection of A-425619 (30 µmol/kg, i.v.) in a CFA-inflamed rat. Shaded region on the paw represents the neuronal receptive field. *p < 0.05, v.s. baseline firing, +p<0.05, ++p<0.01 v.s. vehicle-treated group.

Figure 4. Systemic injection of A-425619 (30 µmol/kg, i.v.) was more effective in reducing WDR responses to noxious thermal stimulation in CFA-treated than in uninjured rats. n = 5-8 per group, *p < 0.05, v.s. baseline firing, ++p<0.01 v.s. vehicle-treated group, $$p < 0.01 v.s. uninjured group.

Figure 5. (A) Administration of intraplantar A-425619 dose-dependently (30-300 nmol) reduced thermal-evoked WDR activity in both CFA-treated and uninjured rats. The effect was similar between the two groups (n = 5-9 per group). (B) Representative ratemeter showing the thermal-evoked activity of a single WDR neuron both before (base) and after
intraplantar injection of A-425619 (300 nmol) in an uninjured rat. Shaded region on the paw represents the neuronal receptive field. *p < 0.05, v.s. baseline firing, +p<0.05, ++p<0.01 v.s. vehicle-treated group.

Figure 6. (A) Administration of A-425619 (20 nmol) into the L5 DRG reduced the number of thermal-evoked WDR discharges in both uninjured and CFA-inflamed rats. However, the effect was significantly greater in the inflamed animals (n = 6-9 per group). (B) Representative ratemeter showing the thermal-evoked activity of a single WDR neuron both before (base) and after the intra-DRG injection of A-425619 (20 nmol) in a CFA-inflamed rat. Shaded region on the paw represents the neuronal receptive field *p < 0.05, vs. baseline firing, +p<0.05 vs. vehicle-treated group, $p< 0.05 vs. uninjured group.

Figure 7. Administration of 5% lidocaine into spinal tissue (in 0.2 µl) more potently reduced thermal-evoked and spontaneous WDR firing than injection onto the L5 DRG (in 2.0 µl). Spontaneous firing was almost completely shut down by the intra-spinal injection of lidocaine, but was not significantly reduced by the intra-DRG injection indicating that the differential effects of lidocaine were site-specific and not due to diffusion between these sites. Data shown are 5 min after injection of lidocaine, n = 12 for intra-DRG injections (7 CFA and 5 uninjured rats), n = 5 for intra-spinal injections (3 CFA and 2 uninjured rats); *p<0.05 vs. vehicle-treated group, $$p<0.01 vs. DRG injection group.
Figure 6. (A) Systemic administration of A-425619 did not alter the spontaneous firing of spinal WDR neurons. (B) Representative ratemeter showing the activity of a single neuron over the entire recording period (about 60 min). While thermal-evoked activity (H) decreased following the systemic administration of A-425619 (30 µmol/kg, i.v.) in this CFA-inflamed rat, spontaneous activity was unaffected. Baseline spontaneous activity is measured during the first 5 min of the experiment. Shaded region on the paw represents the neuronal receptive field; P = noxious pinch, dry = drying the paw after removal from the water.

Figure 7. Intraplantar injection of A-425619 (100 nmol) completely blocked the responses of WDR neurons to capsaicin (1 µg) stimulation. At the highest dose tested (30 µmol/kg, i.v.), systemic administration of A-425619 significantly attenuated, but did not block the capsaicin-evoked WDR activity. n = 5-7 per group. +p<0.05, ++p<0.01 v.s. vehicle-treated group.
Fig 1

![Chemical Structure](image-url)
Fig 2.
Fig 3.

A

% of baseline heat evoked activity (spikes/15 sec)

Dose (μmol/kg, i.v.)

B

spikes/sec

Base 5 15 25

time after A-425619 (min)

10 s
Fig 4
Fig 5.
Fig 6.
Fig 7.
Figure 8
Figure 9