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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McGaraughty, Steve, Katharine L. Chu, Connie R. Faltynek, and Michael F. Jarvis. Systemic and site-specific effects of A-425619, a selective TRPV1 receptor antagonist, on wide dynamic range neurons in CFA-treated and uninjured rats. J Neurophysiol 95: 18–25, 2006. First published September 14, 2005; doi:10.1152/jn.00560.2005. 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. To further understand the peripheral mechanisms of TRPV1-related antinociception, we examined the effects of systemic and site-specific injections of A-425619 on evoked and spontaneous firing of spinal wide dynamic range (WDR) neurons in uninjured rats and rats with peripheral inflammation (CFA; 48 h). 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, iv) 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 intradorsal 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 after intraplantar injection, the effects of A-425619 after intra-DRG injection were enhanced in the inflamed rats (compared with 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 signaling to WDR neurons after injury may be the result of changes to the distribution/sensitization of peripheral TRPV1 receptors.

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 (Di Marzo 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 (Guo et al. 1999; Valtschanoff 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; Roberts et al. 2004; Szabo et al. 2002). The primary afferent TRPV1 receptor is postulated to be a molecular integrator because of 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-arachidonoylethanolamide (NADA), and lipoxigenase derivatives may activate TRPV1 receptors located both inside and outside of the CNS (Di Marzo et al. 2002; Huang et al. 2002; Hwang et al. 2000; Sagar et al. 2004; Zygmont 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). After 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 with 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 after 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 recently been reported that systemic administration of A-425619 (Fig. 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 CNS when administered systemically and was most effective to reduce thermal hyperalgesia after a chronic inflammatory injury caused by intraplantar administration of complete Freund’s adjuvant (CFA) (Honore et al. 2005). To further understand the peripheral mechanisms of TRPV1-related antinociception, we examined the effects of systemic A-425619 on evoked and spontaneous firing of spinal wide dynamic range (WDR) neurons in uninjured and CFA-inflamed rats. Furthermore, we studied the relative contributions of TRPV1 receptors located on periph...
eral terminals and the dorsal root ganglion (DRG) to the effects of A-425619.

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, 350–400 g) were used for all experiments and were housed in a temperature controlled room with a 12/12-h day/night cycle. Food and water were available ad libitum.

To induce chronic inflammatory hyperalgesia, a 150-μl solution of 1:1 CFA and PBS was injected subcutaneously into the plantar region of the rat’s right hindpaw 48 h before testing. On the day of neuronal recording, CFA-inflamed and naïve animals were initially anesthetized with pentobarbital (50 mg/kg, ip). 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 clamps attached to the vertebral processes on either side of the exposure site. The exposed lumbar area was enveloped by agar and clamps attached to the vertebral processes on either side of the exposure site. The exposed lumbar area was enveloped by agar and 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/h (iv). Body temperature was kept at ~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 to 52°C. Spike waveforms were monitored on an oscilloscope throughout the experiment, digitized (32 points), and 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 five 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 characterized by their responses to innocuous (tap, brush, air puff) and noxious stimuli (pinch, heat) applied to the right hindpaw. The hindpaw receptive field to pinch stimulation was subsequently mapped for each neuron. The actions of A-425619 on WDR neuronal activity were 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 noncirculating 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 (Poly-science, Niles, IL). To produce similar levels of baseline evoked firing, uninjured and CFA-treated animals received different intensities of thermal stimulation. Because 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, whereas the hindpaw of CFA-injected rats was immersed in 47°C water for 10 s. As a measure of baseline (predrug) 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 administered systemically (3–30 μmol/kg, iv), intraplantarly (30–300 nmol in 50 μl), or directly onto the L5 DRG (20 nmol in 2 μl). Thermal-evoked and spontaneous activity was 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 sitespecific 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 the spinal tissue (0.2 μl) using a technique described previously (Heinricher and McGaraughty 1998). Briefly, for the latter protocol, a glass infusion pipette (75–80 μm OD) with an angled beveled tip was attached to the recording electrode in such a way that the tips were separated by ~300 μm laterally and 30–100 μm dorsoventrally. The electrode and pipette were simultaneously 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, iv) or intraplantarly (3–100 nmol in 50 μl). Capsaicin (1 μg in 10 μl) was 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 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 postdrug spontaneous and thermal-evoked activity was calculated as a percent of their respective baseline levels. All data are presented as means ± SE. For comparisons to baseline firing
levels, statistical significance was established by using a Wilcoxon’s matched-pairs test. A Kruskal-Wallis ANOVA 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. Because 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 $788.1 \pm 20.8 \mu m$ from the surface of the spinal cord. Baseline (predrug) 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 (Mainxner 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 (Fig. 2). 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, whereas 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. tk;2Systemic administration of A-425619 (3–30 μmol/kg, iv) dose-dependently attenuated thermal-evoked WDR activity in CFA-treated rats with an eED$_{50}$ of $\sim 10 \mu mol/kg$ (Fig. 3A). The systemic effect occurred within 5 min of injection and lasted for the duration of the recording period (Fig. 3B). The effect of A-425619 (30 μmol/kg, iv) 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; Fig. 4). These effects of systemic A-425619 were not related to an effect on blood pressure because the achieved plasma concentrations ($\sim 14.1 \text{ng/ml}$) of the highest dose tested, 30 μmol/kg (iv), 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 observations). 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 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 with baseline ($P < 0.01$). By the next stimulus presentation at 15 min after vehicle injection, the mean evoked response to thermal stimulation was still slightly elevated but was not significantly different from baseline levels. Similar effects on evoked activity in CFA-inflamed rats have been observed with an intraplantar injection of a 100% saline vehicle (McGarughty and Chu, unpublished observations), suggesting that this was caused by needle insertion and injection of a 50-μl volume into the neuronal receptive field of a sensitized paw and not because of 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 (Fig. 5). The efficacy of intraplantar A-425619 was similar between these two groups as the eED$_{50}$’s 15 min after injection were 75 (uninjured rats) and 85 nmol (CFA-treated rats). The significant antihyperalgesic effect of A-425619 lasted for the duration of the recording period (25 min after injection) in both CFA-inflamed and uninjured rats (Fig. 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 (Fig. 6). The significant antihyperalgesic 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 (Fig. 6B) and uninjured rats.

INTRA-DRG OR INTRASPINAL ADMINISTRATION OF LIDOCAINE. Possible diffusion of drug from the DRG to relevant spinal tissue using the current intra-DRG technique was studied by comparing the effects of lidocaine on WDR neuronal activity after intra-DRG or intraspinal injection. In both CFA and

![Fig. 2](http://jn.physiology.org/)

FIG. 2. Wide dynamic range (WDR) responses to increasing stimulus temperature in complete Freund’s adjuvant (CFA) and uninjured rats anesthetized with a continuous infusion of propofol. Shaded region on the paw represents neuronal receptive field.
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 (Fig. 7). However, the intraspinal 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 after the spinal injection of lidocaine but was not significantly affected by the intra-DRG injection (Fig. 7). This latter finding, showing a lack of significant effects on spontaneous firing after the intra-DRG injection, suggests that using this 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 (Fig. 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 to 1) evaluate A-425619’s efficacy against a specific TRPV1-mediated inflammation 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 pretreatment with intraplantar A-425619 (100 nmol; Fig. 9). Furthermore, the eED50 (3–100 nmol) to attenuate capsaicin-evoked WDR firing after intraplantar injection of A-425619 was ~40 nmol (data not shown). Using the highest dose administered systemically to affect thermal-evoked activity in uninjured rats, pretreatment with 30 μmol/kg (iv) of A-425619 reduced capsaicin-evoked activity by only 56% (145 ± 47.7 spikes; P < 0.05) compared with the vehicle group (Fig. 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 8 h after injection (El Kouhen et al. 2005; Honore et al. 2005). The antinociceptive actions of A-425619 were likely mediated by peripheral sites because A-425619 does not readily enter the CNS after 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, are consistent with the compound’s antihyperalgesic 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 after an inflammatory injury.

The apparent increase in TRPV1-related use of WDR neurons after injury may be the result of changes to the distribution of TRPV1 receptors. Under normal physiological conditions, TRPV1 receptors are localized to C- and Aδ-primary afferent fibers, and the distribution is weighted toward C-fibers (Caterina et al. 2000). After a chronic injury, including CFA-induced inflammation, expression of TRPV1 receptors has been shown to increase to a much...
greater degree in the myelinated Aδ- and Aβ-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, endovaniloids, and TRPV1-sensitizing agents an additional or alternate means to affect neuronal activity in the spinal dorsal horn after injury. These data suggest that A-425619 blocked this additional input from reaching WDR neurons in the inflamed rats. Although there is some evidence that Aβ-fibers have a “phenotype switch” after an inflammatory injury that affects spinal hypersensitivity (Neumann et al. 1996), and that these large diameter afferents are sensitive to temperature change after injury (Li et al. 2002), the redistribution of TRPV1 to Aδ-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 these 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 shown 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 this 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. Because of 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 this 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. Because 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 (2nd) phase of the formalin assay (Honore et al. 2005), an outcome that is consistent with a lack of effect on central sensitization (Coderre et al. 1990; Dickenson and Sullivan...
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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 that parallels the actions of systemic A-425619. This latter outcome is also consistent with the upregulation and redistribution of TRPV1 receptors in the DRG after 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 after systemic delivery through the site’s 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 Coggshall 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 physicochemical 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-425619 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 after systemic injection, at the highest dose tested (30 μmol/kg), were probably much lower than those achieved after intraplantar administration (100 nmol). Consistent with this hypo-

FIG. 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 dorsal root ganglion (DRG; in 2.0 μl). Spontaneous firing was almost completely shut down by the intraspinal 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 caused by 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 intraspinal injections (3 CFA and 2 uninjured rats). *P < 0.05 vs. vehicle-treated group, $P < 0.01 vs. DRG injection group.

1987). Even though the antinoceptive 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 therapeutically 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, because 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 effects of systemic A-425619 on WDR activity 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 TRPV1 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 that parallels the actions of systemic A-425619. This latter outcome is also consistent with the upregulation and redistribution of TRPV1 receptors in the DRG after 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 after systemic delivery through the site’s ample blood supply.

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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-425619 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 after systemic injection, at the highest dose tested (30 μmol/kg), were probably much lower than those achieved after intraplantar administration (100 nmol). Consistent with this hypo-

FIG. 8. 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 (~60 min). While thermal-evoked activity (H) decreased after systemic administration of A-425619 (30 μmol/kg, iv) in this CFA-inflamed rat, spontaneous activity was unaffected. Baseline spontaneous activity is measured during the 1st 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.
thesis, whereas intraplantar administration of A-425619 (100 nmol) completely blocked capsaicin-evoked WDR activity, systemic delivery of A-425619 (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 after 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.

REFERENCES


