Published in final edited form as:
Exp Neurol. 2007 March ; 204(1): 454–457.

Antinociceptive effect of cannabinoid agonist WIN 55,212–2 in rats with a spinal cord injury

Aldric Hama* and **Jacqueline Sagen**
University of Miami Miller School of Medicine, The Miami Project to Cure Paralysis, Miami, FL 33136, USA

Abstract

Spinal cord injury (SCI) pain exhibits many symptoms associated with peripheral neuropathic pain, including increased tactile hypersensitivity. One novel approach to ameliorate SCI pain is the use of cannabinoid (CB) ligands. The current study evaluated the efficacy of the nonselective CB receptor agonist WIN 55,212–2 on tactile hypersensitivity in rats following a brief compression to the thoracic spinal cord. The withdrawal thresholds of the hind paws following SCI were significantly decreased, indicating tactile hypersensitivity. Systemic injection of WIN 55,212–2 increased withdrawal thresholds in a dose-dependent manner. Pretreatment with the CB_1 receptor subtype-selective antagonist AM 251 completely abolished the antinociceptive effect of WIN 55,212–2 whereas pretreatment with the CB_2 receptor subtype-selective antagonist AM 630 did not alter the antinociceptive effect of WIN 55,212–2. These data indicate that a CB_1 selective agonist may be novel therapeutic treatment for clinical SCI pain.

Keywords

AM 251; AM 630; allodynia; chronic pain; CB_1 receptor; neuropathic pain

Trauma or disease to either the peripheral or central nervous system leads to persistent pain. There are few effective treatments for patients with chronic neuropathic pain. The cannabinoids show promise in alleviating peripheral neuropathic pain, which in turn, may have efficacy on central neuropathic pain states.

Studies in rats indicate that the cannabinoid receptors (subtypes CB_1 and CB_2) have key roles in modulating pain, especially neuropathic pain. The CB_1 receptor has been identified in the rat dorsal root ganglia, spinal cord, and brain areas relevant to the processing of pain-related information (Farquhar-Smith et al., 2000; Hohmann et al., 1999; Tsou et al., 1998). There are numerous studies in rat models of peripheral neuropathic pain that demonstrate significant suppression of thermal and mechanical hypersensitivity with a non-selective CB receptor agonist, which is attenuated with a selective CB_1 receptor antagonist (Bridges et al., 2001; Fox et al., 2001; Herzberg et al., 1997; Pascual et al., 2005; Ulugol et al., 2004). However, a role for the CB_1 receptor in rats with central neuropathic pain caused by a spinal cord injury (SCI) has not been examined. The current study evaluated the effect of the non-selective CB receptor agonist WIN 55,212–2 in a rat model of neuropathic pain induced by compression of the spinal

*Corresponding author: Aldric Hama, Ph.D., University of Miami Miller School of Medicine, The Miami Project to Cure Paralysis, 1095 NW 14th Terrace (R-48), Miami, FL 33136, USA, Telephone: 305-243-6038, Facsimile: 305-243-3923, E-mail: ahama@med.miami.edu.

cord. To determine the receptor subtype that mediates the effect of WIN 55,212–2, rats were pretreated with either AM 251, an analogue of the CB_1 receptor selective antagonist SR 141716A, or AM 630, a CB_2 receptor selective antagonist (Gatley et al., 1996; Pertwee et al., 1995).

Surgical and behavioral testing procedures were reviewed and approved by the University of Miami Animal Care and Use Committee. Care of rats followed the guidelines of the National Institutes of Health. Male Sprague Dawley rats (Harlan, IN; 125–150 g at the time of surgery) were used. A modified procedure was used to induce a spinal cord injury (Bruce et al., 2002). Under isoflurane anesthesia, rats were rendered unconscious and the back was shaved and swabbed with povidone iodine. Using aseptic surgical technique, a 1 mm segment of thoracic spinal cord at a level between T6 and T8 was exposed via a laminectomy and compressed for one minute with a micro-aneurysm clip which exerted 20 g of force (Harvard Apparatus, MA). The back muscles were sutured closed and the skin sealed with wound clips. Rats were allowed to recover in their home cages and had free access to food and water. Bladder function in these rats returned about 1–2 days following surgery.

The hind paw response to innocuous mechanical stimulation was tested with von Frey filaments. Rats were placed in Plexiglas cages which rested on an elevated wire mesh floor. A series of filaments was applied to the center of the plantar left hind paw and the pattern of responses (withdrawal of the hind paw from the filament) to the filaments were converted into a 50% withdrawal threshold (reported in grams) (Chaplan et al., 1994). Following testing of the left hind paw, the right hind paw was tested.

Hind limb function diminished from a pre-surgery score of 21 to about 11 on the Basso, Beattie, and Bresnahan locomotor rating scale (data not shown; (Basso et al., 1995)). A score of 11 indicates weight bearing on the hind paws and a lack of hind paw coordination with the fore paws during locomotion. Hind limb functionality remained at this decreased level for at least 5 weeks post-surgery but it was still possible to evoke hind paw responses with von Frey filaments. A robust hind paw mechanical hypersensitivity was observed beginning one week following spinal cord compression surgery, which persisted for at least 5 weeks, so rats were tested 4–5 weeks after surgery. A withdrawal threshold of 4 g or less in either hind paw was used as an inclusion criterion for drug testing. A total of 48 rats were used in these studies.

Following baseline withdrawal threshold measurement, 24 rats with a spinal compression were subcutaneously injected with a dose of *R*-(+)-WIN 55,212–2 mesylate (0.3, 1, 3 mg/kg; Tocris, MO) or vehicle (10% DMSO/5% Tween 80/85% saline) in a volume of 1 ml/kg. Rats were then tested once every 30 min for 120 min following injection. Rats were humanely euthanized following testing.

To determine the CB receptor subtype responsible for the antinociceptive effect of WIN 55,212–2, 24 rats (12 rats per antagonist treatment) were subcutaneously injected with either AM 251 (3 mg/kg; Tocris), AM 630 (1 mg/kg, Tocris) or vehicle, in a volume of 2 ml/kg. Following baseline withdrawal threshold measurement, rats were injected with either antagonist or vehicle. Thresholds were again measured 30 min after the injection of either antagonist or vehicle. Rats were then injected with either 3 mg/kg WIN 55,212–2 or vehicle and were tested 30 min later. Thus, there were four treatment groups in the antagonist phase of the experiment (pre-treatment/post-treatment): vehicle/vehicle, antagonist/vehicle, vehicle/WIN 55,212–2 and antagonist/WIN 55,212–2. The rats were used twice in the antagonist experiments in a psudo-Latin square design, being allowed a two day washout period in between tests. Rats were euthanized after the second round of testing.

Data are expressed as mean ± S.E.M. The effect of the doses of WIN 55,212–2 over time was statistically analyzed with a repeated-measure two-way ANOVA, followed by a Student-

Newman-Keuls test for *post hoc* comparisons. The 50% analgesic dose was calculated from the dose-response curve 30 min after injection (Tallarida and Murray, 1981). The data of the antagonists with WIN 55,212–2 were statistically analyzed using a two-way ANOVA followed by a Student-Newman-Keuls test for *post hoc* comparisons. Statistical significance was taken at $p < 0.05$.

Effect of WIN 55,212–2 on tactile hypersensitivity over time

The mean withdrawal threshold of naïve rats prior to spinal compression surgery was 15 ± 0.0 g (data not shown). Prior to injection of either WIN 55,212–2 or vehicle, the withdrawal threshold of all SCI rats was 1.7 ± 0.2 g (Fig. 1). Robust dose-dependent increases in withdrawal thresholds, indicating significant reversal of mechanical hypersensitivity, were observed with 1 and 3 mg/kg WIN 55,212–2 ($p < 0.05$ vs. baseline). Increased withdrawal thresholds with these doses were observed 30 min after injection, which lasted throughout the observation period ($p < 0.05$ vs. vehicle at all time points). By contrast, injection of either 0.3 mg/kg WIN 55,212–2 or vehicle did not significantly alter withdrawal thresholds. The 50% analgesic dose of WIN 55,212–2 30 min after injection was 0.7 (95% C.L. = 0.5–1.0) mg/kg.

Effect of cannabinoid antagonists on the antinociceptive effect of WIN 55,212–2

The mean withdrawal threshold of naïve rats prior to spinal compression surgery was 15 ± 0.0 g (data not shown). Prior to injection of either AM 251 or vehicle, the withdrawal threshold of all rats was 2.3 ± 0.1 g (Fig. 2A). Injection of AM 251 (or vehicle) did not affect withdrawal thresholds when compared with pre-injection thresholds ($p > 0.05$). Pretreatment with AM 251 blocked the onset of the antinociceptive effect of WIN 55,212–2. Thirty minutes after injection of WIN 55,212–2, the withdrawal threshold of the AM 251/WIN group (2.1 ± 0.4 g) was significantly less than that of the vehicle/WIN group (15 ± 0.0 g; $p < 0.05$) and was not different from the threshold of the vehicle/vehicle group (2.4 ± 0.3 g; $p > 0.05$).

Prior to injection of either AM 630 or vehicle, the withdrawal threshold of all rats was 2.2 ± 0.2 g (Fig. 2B). Injection of AM 630 (or vehicle) did not affect withdrawal thresholds when compared with pre-injection thresholds ($p > 0.05$). Pretreatment with AM 630 did not significantly alter the antinociceptive effect of WIN 55,212–2. Thirty minutes after injection of WIN 55,212–2, the withdrawal threshold of the AM 630/WIN group (14.4 ± 0.6 g) was not significantly different from the withdrawal threshold of the vehicle/WIN group (15 g; $p > 0.05$), but was significantly different from the threshold of the vehicle/vehicle group (2.1 ± 0.2 g; $p < 0.05$) and AM 630/vehicle group (2.9 ± 0.4 g; $p < 0.05$).

The current study is the first to demonstrate an antinociceptive effect of a nonselective CB receptor agonist in a rat model of SCI pain. In addition, the antinociceptive effect of WIN 55,212–2 is mediated through the CB_1 receptor. The current data are in agreement with previous studies that have shown antinociceptive activity of CB receptor agonists in peripheral neuropathic pain models and strongly support the notion that a CB_1 receptor agonist has potential as an analgesic for clinical SCI pain.

There are a number of studies that have demonstrated the efficacy of nonselective CB agonists in rat models of chronic neuropathic pain (Bridges et al., 2001; Fox et al., 2001; Herzberg et al., 1997; Pascual et al., 2005). These studies, in which WIN 55,212–2 was tested on a variety of sensory modalities, also point out that the antinociceptive effect is due mostly, if not entirely, to CB_1 receptor activation since SR 141716A completely attenuated the antinociceptive effect. The 50% antinociceptive dose in the current study was 0.7 mg/kg, which is close to a previously reported 50% antinociceptive dose of 0.52 mg/kg, even though the sensory test in the previous

report was entirely different (Fox et al., 2001). Similar to previous reports, the effect of WIN 55,212–2 in the present study on mechanical hypersensitivity was suppressed by a pretreatment with AM 251, an analog of SR 141716A. The current results extend previous findings, in that the compound was tested in a strikingly different model – a model of central neuropathic pain.

Interestingly, a few studies suggest that CB_2 receptors may also be involved in the effect of these nonselective CB receptor agonists on peripheral neuropathic allodynia (increased responsiveness to non-noxious stimuli) (Ibrahim et al., 2003; Scott et al., 2004). Following peripheral nerve injury, CB_2 receptors are upregulated in spinal microglia (Zhang et al., 2003). However, the role of CB_2 receptor-expressing microglia in neuropathic pain, specifically in SCI pain, remains to be determined. Alternatively, it is possible that some selective CB_2 receptor antagonists have inverse agonist as well as antagonist properties, such that the observed “antagonism” is really an increase in pain caused by the “antagonist” (Landsman et al., 1998; Portier et al., 1999; Ross et al., 1998). Finally, it may be the case that CB_2 receptor activity is found in some but not all neuropathic pain models. The current data demonstrates that the CB_2 receptor in SCI pain is not crucial to the antinociceptive effect of WIN 55,212–2 and further suggests a pharmacological differentiation between central vs. peripheral neuropathic pain. Whether this differentiation holds for other receptor classes needs is not known, but if it does, this would support the use of specific analgesic treatments depending on the neuropathy.

Spinal cord injury patients, similar to peripheral neuropathy pain patients, suffer from evoked and unevoked pain. In addition, pain may occur above, at or below the level of the lesion. It has been suggested that the different levels of pain in SCI patients have different etiologies. For example, at-level pain may be due to a combination of abnormal peripheral and central mechanisms, whereas below level pain due mostly to abnormal central processing of pain (Finnerup et al., 2003). Indeed, other pain-related symptoms not evaluated in the current study may involve the CB_2 receptor. Currently, the neuroanatomical location of CB receptors in the SCI model is not known. Finding these receptors may facilitate understanding the mechanism of antinociception of this class of compound.

Although not systematically quantified in the current study, rats that received 3 mg/kg WIN 55,212–2 appeared moderately sedated, with less spontaneous activity, compared to vehicle-treated rats. Adverse side-effects were less apparent with the antinociceptive dose of 1 mg/kg, which is consistent with previous studies that quantified body temperature, catalepsy and motor dysfunction following injection of CB agonists (Fox et al., 2001; Pascual et al., 2005). Rats in the AM 251/WIN, but not the AM 630/WIN, group appeared to have normal body posture and activity. This qualitative observation implies that these physiological parameters are mediated by the CB_1, and not the CB_2, receptor (Fox et al., 2001). Unwanted side-effects will be a challenge that will need to be addressed in the development of a CB_1 agonist (Campbell et al., 2001).

The current data demonstrates a role for the CB_1 receptor in experimental SCI pain and suggests evaluating CB_1 receptor agonists for the treatment of clinical SCI pain. A review of the clinical literature regarding the use of presently available nonselective CB ligands in chronic pain, including some types of neuropathic pain, suggests that their efficacy is no better than codeine (Attal et al., 2004; Campbell et al., 2001). Although other studies indicate efficacy in neuropathic pain, the analgesic effect of the drug may be due to a combination of non-CB as well as CB receptor activity (Karst et al., 2003). For central neuropathic pain in particular, one double-blind placebo controlled study using a nonselective CB ligand was done, but neuropathic-related symptoms were not specifically evaluated (Rog et al., 2005). Nevertheless, SCI patients with chronic pain have reported substantial pain relief from marijuana, suggesting the possibility that cannabinoids may be of particular value as a treatment option for this

indication (Cardenas and Jensen, 2006). As alluded to earlier, the somnolence and psychotropic side-effects associated with nonselective CB ligands may not appeal to all chronic pain patients (Campbell et al., 2001; Svendsen et al., 2004). These issues only highlight the need for a well characterized and selective ligand to directly answer the question of whether CB_1 receptor agonists can attenuate neuropathic pain in general and specifically, SCI pain.

Acknowledgements

Supported by NIDA grant DA10546 and by the Miami Project to Cure Paralysis.

References

Attal N, Brasseur L, Guirimand D, Clermond-Gnamien S, Atlami S, Bouhassira D. Are oral cannabinoids safe and effective in refractory neuropathic pain? Eur J Pain 2004;8:173–177. [PubMed: 14987627]

Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 1995;12:1–21. [PubMed: 7783230]

Bridges D, Ahmad K, Rice AS. The synthetic cannabinoid WIN55,212–2 attenuates hyperalgesia and allodynia in a rat model of neuropathic pain. Br J Pharmacol 2001;133:586–594. [PubMed: 11399676]

Bruce JC, Oatway MA, Weaver LC. Chronic pain after clip-compression injury of the rat spinal cord. Exp Neurol 2002;178:33–48. [PubMed: 12460606]

Campbell FA, Tramer MR, Carroll D, Reynolds DJ, Moore RA, McQuay HJ. Are cannabinoids an effective and safe treatment option in the management of pain? A qualitative systematic review. Bmj 2001;323:13–16. [PubMed: 11440935]

Cardenas DD, Jensen MP. Treatments for chronic pain in persons with spinal cord injury: A survey study. J Spinal Cord Med 2006;29:109–117. [PubMed: 16739554]

Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63. [PubMed: 7990513]

Farquhar-Smith WP, Egertova M, Bradbury EJ, McMahon SB, Rice AS, Elphick MR. Cannabinoid CB (1) receptor expression in rat spinal cord. Mol Cell Neurosci 2000;15:510–521. [PubMed: 10860578]

Finnerup NB, Gyldensted C, Nielsen E, Kristensen AD, Bach FW, Jensen TS. MRI in chronic spinal cord injury patients with and without central pain. Neurology 2003;61:1569–1575. [PubMed: 14663044]

Fox A, Kesingland A, Gentry C, McNair K, Patel S, Urban L, James I. The role of central and peripheral Cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. Pain 2001;92:91–100. [PubMed: 11323130]

Gatley SJ, Gifford AN, Volkow ND, Lan R, Makriyannis A. 123I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors. Eur J Pharmacol 1996;307:331–338. [PubMed: 8836622]

Herzberg U, Eliav E, Bennett GJ, Kopin IJ. The analgesic effects of R(+)-WIN 55,212–2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. Neurosci Lett 1997;221:157–160. [PubMed: 9121688]

Hohmann AG, Tsou K, Walker JM. Cannabinoid suppression of noxious heat-evoked activity in wide dynamic range neurons in the lumbar dorsal horn of the rat. J Neurophysiol 1999;81:575–583. [PubMed: 10036261]

Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, Vanderah TW, Lai J, Porreca F, Makriyannis A, Malan TP Jr. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci U S A 2003;100:10529–10533. [PubMed: 12917492]

Karst M, Salim K, Burstein S, Conrad I, Hoy L, Schneider U. Analgesic effect of the synthetic cannabinoid CT-3 on chronic neuropathic pain: a randomized controlled trial. JAMA 2003;290:1757–1762. [PubMed: 14519710]

Landsman RS, Makriyannis A, Deng H, Consroe P, Roeske WR, Yamamura HI. AM630 is an inverse agonist at the human cannabinoid CB1 receptor. Life Sci 1998;62:PL109–113. [PubMed: 9496703]

Pascual D, Goicoechea C, Suardiaz M, Martin MI. A cannabinoid agonist, WIN 55,212–2, reduces neuropathic nociception induced by paclitaxel in rats. Pain 2005;118:23–34. [PubMed: 16213089]

Pertwee R, Griffin G, Fernando S, Li X, Hill A, Makriyannis A. AM630, a competitive cannabinoid receptor antagonist. Life Sci 1995;56:1949–1955. [PubMed: 7776818]

Portier M, Rinaldi-Carmona M, Pecceu F, Combes T, Poinot-Chazel C, Calandra B, Barth F, le Fur G, Casellas P. SR 144528, an antagonist for the peripheral cannabinoid receptor that behaves as an inverse agonist. J Pharmacol Exp Ther 1999;288:582–589. [PubMed: 9918562]

Rog DJ, Nurmikko TJ, Friede T, Young CA. Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. Neurology 2005;65:812–819. [PubMed: 16186518]

Ross RA, Brockie HC, Fernando SR, Saha B, Razdan RK, Pertwee RG. Comparison of cannabinoid binding sites in guinea-pig forebrain and small intestine. Br J Pharmacol 1998;125:1345–1351. [PubMed: 9863666]

Scott DA, Wright CE, Angus JA. Evidence that CB-1 and CB-2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat. Pain 2004;109:124–131. [PubMed: 15082134]

Svendsen KB, Jensen TS, Bach FW. Does the cannabinoid dronabinol reduce central pain in multiple sclerosis? Randomised double blind placebo controlled crossover trial. Bmj 2004;329:253. [PubMed: 15258006]

Tallarida, RJ.; Murray, RB. Manual of Pharmacological Calculations with Computer Programs. Springer-Verlag; New York: 1981.

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 1998;83:393–411. [PubMed: 9460749]

Ulugol A, Karadag HC, Ipci Y, Tamer M, Dokmeci I. The effect of WIN 55,212–2, a cannabinoid agonist, on tactile allodynia in diabetic rats. Neurosci Lett 2004;371:167–170. [PubMed: 15519750]

Zhang J, Hoffert C, Vu HK, Groblewski T, Ahmad S, O'Donnell D. Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. Eur J Neurosci 2003;17:2750–2754. [PubMed: 12823482]

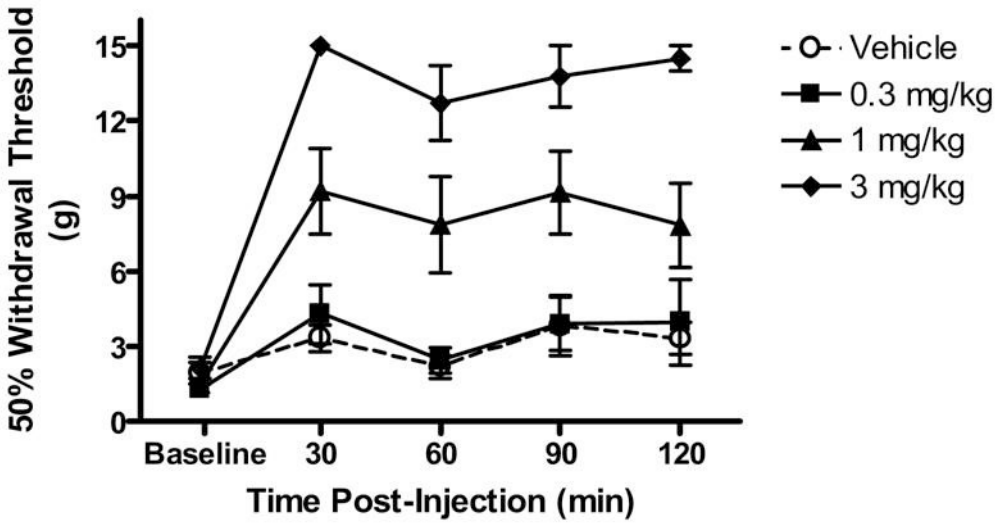

Figure 1.
Effect of WIN 55,212–2 on tactile hypersensitivity over time in rats with a spinal cord compression. Rats were subcutaneously injected with either vehicle or a dose of WIN 55,212–2 and tested once every 30 min. WIN 55,212–2 dose-dependently increased withdrawal thresholds. Data are expressed as mean ± S.E.M., n = 6/group.

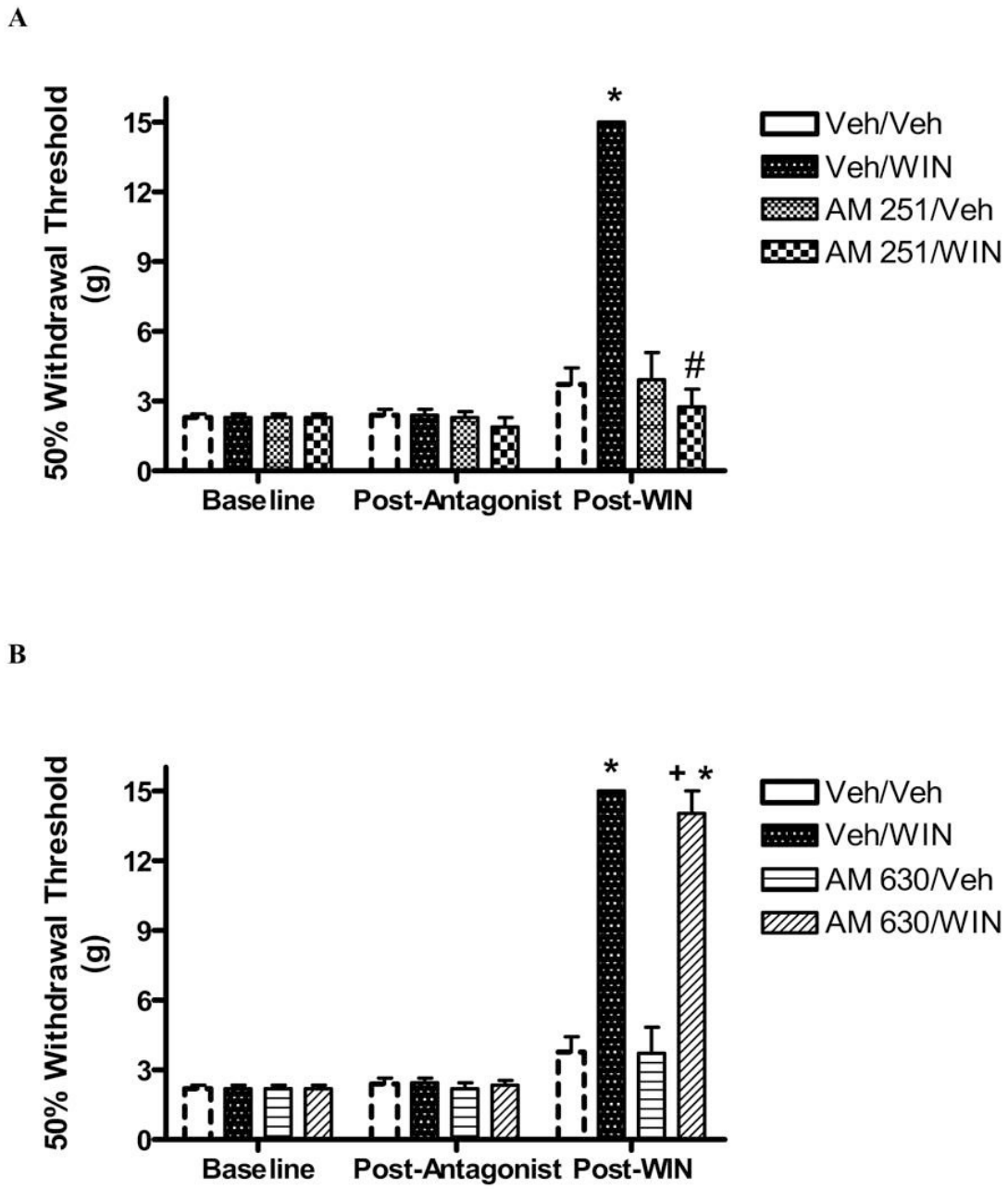

Figure 2.

Effect of cannabinoid antagonists on the antinociceptive effect of WIN 55,212–2 in rats with a spinal cord compression. Following "Baseline" testing, rats were subcutaneously injected with either antagonist or vehicle. Thirty minutes following either antagonist or vehicle injection, withdrawal thresholds were measured ("Post-Antagonist") and then rats were injected with either vehicle or 3 mg/kg WIN 55,212–2. Rats were tested 30 min after WIN 55,212–2 or vehicle injection ("Post-WIN"). **A**) Pre-treatment with 3 mg/kg AM 251 blocked the onset of WIN 55,212–2 antinociception. The treatment groups are: vehicle/vehicle (Veh/Veh), AM 251/vehicle (AM 251/Veh), vehicle/WIN 55,212–2 (Veh/WIN) and AM 251/WIN 55,212–2 (AM 251/WIN). **B**) Pre-treatment with 1 mg/kg AM 630 did not affect the onset of

WIN 55,212–2 antinociception. The treatment groups are: vehicle/vehicle (Veh/Veh), AM 630/vehicle (AM 630/Veh), vehicle/WIN 55,212–2 (Veh/WIN) and AM 630/WIN 55,212–2 (AM 630/WIN). Data are expressed as mean ± S.E.M., n = 6/group. *$p < 0.05$ vs. Veh/Veh, #$p < 0.05$ vs. Veh/WIN, + $p < 0.05$ vs. AM630/Veh.