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Sustained antinociceptive effect of cannabinoid receptor agonist WIN 55,212-2 over time in rat model of neuropathic spinal cord injury pain

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Abstract

A significant complaint associated with spinal cord injury (SCI) is chronic pain, which includes symptoms such as cutaneous hypersensitivity and spontaneous unevoked pain and is difficult to treat with currently available drugs. One complication with current analgesics is tolerance, a decrease in efficacy with repeated treatment over time. One promising class of pharmacological treatment is cannabinoid (CB) receptor agonists. The current study assessed the efficacy of the CB receptor agonist WIN 55,212-2 (WIN) in a rat model of neuropathic SCI pain. Brief spinal compression leads to significant hindpaw hypersensitivity to tactile stimulation. WIN dose-dependently increased withdrawal thresholds and continued to demonstrate efficacy over a twice-daily 7-day treatment regimen. By contrast, the efficacy of morphine in SCI rats decreased over the same treatment period. Similarly, the antinociceptive efficacy of WIN to acute noxious heat in uninjured rats diminished over time. These data suggest that the sustained efficacy of a CB receptor agonist for pain could depend on the pain state. Such agonists may hold promise for long-term use in alleviating chronic SCI pain.

Keywords

allodynia; alternative medicine; chronic pain; natural product; neuropathic pain; opiate; rat model; rehabilitation; spinal cord injury; tolerance

INTRODUCTION

The incidence of spinal cord injury (SCI) is about 3 percent of all combat-related wounds [1–2]. High mortality rates following SCI were observed in previous military conflicts, but recent advances in emergency medicine and improved rehabilitation have increased patient survival [3]. In addition to physical disability and psychological distress, a significant complication accompanying SCI is moderate to severe intractable pain [4–8]. The prevalence of pain in veterans and nonveterans with SCI is similar (~70%) [4]. However, veterans report both a higher average pain rating and worst-pain rating than nonveterans [4]. As the population of both civilian and veteran SCI patients ages, the need for pain control, in addition to rehabilitation services, will increase [9].

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Although SCI pain may be present at any level relative to the lesion, pain below the lesion has been particularly difficult to treat [6,8,10]. The symptoms of below-level pain are reminiscent of neuropathic pain, which includes hypersensitivity to cutaneous stimulation and diffuse spontaneous pain, variously described as shooting, burning, and electric [4,11–13]. Neuropathic SCI pain treatment options were limited to surgical procedures such as cordotomy and, in an extreme case, bilateral frontal lobotomy [6,10]. In the past, narcotics were denied to patients because of the misguided fear of addiction [8]. Poorly treated SCI pain degrades mood and hinders full participation in rehabilitation and integration into society, which may further heighten pain and anxiety [5]. Thus, nonsurgical, effective SCI pain control is needed.

A promising class of analgesics is ligands that activate the cannabinoid (CB) receptor. Preclinical studies indicate robust antinociception following acute administration of CB receptor agonists in various pain models, including neuropathic SCI pain [14–15]. Several clinical studies have reported a robust analgesic effect in several pain states of Δ^9-Tetrahydrocannabinol (THC), an active component of marijuana and a CB receptor agonist [16–18]. However, it is not clear whether repeated administration will lead to the decrement of antinociceptive efficacy (tolerance) as observed with other drugs (e.g., opiates) [19]. The primary objective of the current study was to evaluate the efficacy over time of daily administration of the CB receptor agonist WIN 55,212-2 ((R)-(+)-[2,3-Dihydro-5-methyl-3 [(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone) (WIN) and morphine in rats with neuropathic SCI pain. Since WIN is known to be antinociceptive in uninjured rats, the effect of WIN over time, using the same treatment schedule as in SCI rats, was also evaluated [20].

METHODS

Animals and Surgery

Male Sprague-Dawley rats (Harlan Laboratories; Indianapolis, Indiana) were used. Rats to be used for spinal cord compression surgery ($N = 39$) were about 100 to 125 g at the time of arrival to the animal facility and housed two per cage. Before and after surgery, rats were allowed free access to water and standard rat chow. Studies were reviewed and approved by the University of Miami Animal Care and Use Committee.

The procedure to induce a spinal cord compression-type injury was described previously [15]. The rat was anesthetized with isoflurane in oxygen and its back was shaved and swabbed with chlorhexidine. With aseptic surgical technique, a laminectomy was performed to expose the sixth to seventh thoracic spinal segment. A microvascular clip (Harvard Apparatus; Holliston, Massachusetts) was placed vertically on the exposed thoracic spinal cord, such that the clip compressed the entire segment, and then left in place for 60 s. Care was taken not to cut the dura or disturb nearby spinal nerve roots. After spinal compression, the clip was removed, the muscles were sutured shut, and the skin was closed with wound clips. Bladder function spontaneously returned in these rats 1 to 2 days after surgery.

Sensory Testing

Mechanical Stimulus—To evaluate hindpaw response to innocuous mechanical stimuli, we measured the withdrawal thresholds (in grams) by the up-down method with von Frey filaments [21]. Before surgery, rats did not respond to the highest force filament (15 g).

Four weeks after spinal compression, hindpaw baseline withdrawal thresholds were measured in rats. Stable hindpaw hypersensitivity was previously observed to occur at this time after surgery [22]. Rats were placed in Plexiglas containers with a wire mesh floor and allowed to

acclimate. For a rat to be included in the study, the withdrawal threshold of one hindpaw had to be 4 g or less.

Following baseline testing, rats were injected subcutaneously with either WIN (0.3, 1, 3 mg/kg in 45% β-hydroxyl-propyl-cyclodextrin in water, 2 mL/kg), morphine sulfate (3 mg/kg in saline, 1 mL/kg), or respective vehicles. Rats were tested 30 min postinjection. Injections of either drugs or vehicle occurred twice daily (BID) about 8 am and 5 pm. Testing was performed after the morning injections. Drugs were obtained from Sigma-Aldrich, Corp (St. Louis, Missouri).

Acute Thermal Stimulus—In uninjured rats (250–275 g at the time of testing; $N = 28$), a baseline response latency (in seconds) to a noxious heat source was measured with a hot plate apparatus (Columbus Instruments; Columbus, Ohio). Rats were placed on a heated surface (55 °C) and the amount of time between placement on the apparatus and a hindpaw lick or jump was recorded. Rats were then injected with either WIN or vehicle and tested 30 min postinjection. To avoid tissue damage due to prolonged exposure to the heated surface, we used a cutoff time of 45 s. Rats were injected BID but tested only after the morning injections.

Data Analysis

The withdrawal thresholds of the hindpaws were used in calculating the percent maximum possible effect (MPE):

$$\%\mathrm{MPE}_{\mathrm{threshold}} = ((\text{Drug threshold} - \text{Baseline threshold}) \div (15\ \mathrm{g} - \text{Baseline threshold})) \times 100. \quad (1)$$

The response latencies from the hot plate test were also converted into a percent MPE:

$$\%\mathrm{MPE}_{\mathrm{latency}} = ((\text{Drug latency} - \text{Baseline latency}) \div (45\ \mathrm{s} - \text{Baseline latency})) \times 100. \quad (2)$$

The MPE values were plotted versus dose-response. From the linear portion of the dose-response curves, the mean MPE of each dose and the 50 percent antinociceptive (A50) dose were calculated using a computer program [23]. The A50 values at day 1 and either day 5 (hot plate test) or day 7 (von Frey filaments) were compared to determine whether a significant change in potency had occurred. A rightward shift in the A50 values at either day 5 or day 7 suggests that the drug effect has diminished over repeated administration (tolerance).

Data are expressed as mean ± standard error of the mean (SEM). Statistical analyses of drug effects over time were performed using a two-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test. Statistical significance was taken at $p < 0.05$.

RESULTS

Spinal Cord Injury Mechanical Hypersensitivity

WIN—Four weeks after spinal compression surgery, the mean ± SEM hindpaw withdrawal thresholds of the WIN group and vehicle group were 1.8 ± 0.2 g and 2.4 ± 0.3 g, respectively (Figure 1(a)). On day 1 of injection, WIN dose-dependently increased withdrawal thresholds 30 min postinjection (Figure 1(b)). The A50 dose (95% confidence interval) was 0.9 (0.6–1.2) mg/kg [15]. The A50 dose of WIN on day 7 was 0.8 (0.6–1.2) mg/kg, which was not significantly different from day 1 (Figure 2). The MPEs of 3 mg/kg of WIN were not significantly different from day 1 (99% ± 1%) and day 7 (88% ± 9%).

Before morning injections, baseline thresholds of the treatment groups did not significantly change over the 7-day treatment period ($p > 0.05$).

The withdrawal threshold in a small group of age-matched control rats was 15 g. These rats did not undergo surgery. Seven days of treatment with either 3 mg/kg of WIN or vehicle did not alter withdrawal thresholds (data not shown).

Morphine—Four weeks after spinal surgery, the preinjection withdrawal thresholds of the morphine group and vehicle group were 2.6 ± 0.2 g and 2.4 ± 0.3 g, respectively (Figure 3(a)). On day 1 of injection, morphine significantly increased withdrawal thresholds 30 min postinjection (Figure 3(b)). By day 3, the efficacy of morphine (MPE = 61% ± 11%) was significantly decreased compared with the efficacy of the first injection (88% ± 7%; $p < 0.05$ vs day 1). By day 7, the MPE of morphine was 38 ± 8 percent ($p < 0.05$ vs day 1). Despite the decrease in peak efficacy, the withdrawal thresholds after morphine treatment were significantly greater than those after vehicle treatment on all days except day 6 ($p < 0.05$ vs vehicle). Preinjection thresholds did not significantly change over time ($p > 0.05$).

Acute Thermal Nociception

The preinjection withdrawal latency of all rats was 11.1 ± 0.5 s (Figure 4(a)). On day 1 of treatment, WIN dose-dependently increased response latencies in the hot plate test (Figure 4 (b)). Peak efficacy was observed 30 min following injection, and the A50 dose was 1.1 (0.6–1.8) mg/kg. By day 5, the MPE of the 3 mg/kg dose markedly decreased to 30 ± 13 percent, compared with the MPE of day 1, 82 ± 12 percent ($p < 0.05$). The A50 dose was also significantly increased, 15.1 (6.2–36.3) mg/kg, a 14-fold rightward shift of the dose-response curve (Figure 5). One should note that even though the efficacy of 3 mg/kg of WIN decreased over time, a significant increase in latency was still observed at day 5 ($p < 0.05$ vs vehicle).

Baseline preinjection latencies did not significantly change over time. At no time did vehicle injection alter latencies.

Neither saline (morphine vehicle) nor β-hydroxyl-propyl-cyclodextrin (WIN vehicle) significantly affected responses to stimuli (Figures 1, 3–4; $p > 0.05$ vs baseline).

DISCUSSION

In rats with neuropathic SCI pain, repeated treatment with the CB receptor agonist WIN resulted in a sustained reduction of neuropathic pain-related behavior. By contrast, repeated treatment with WIN in uninjured rats led to a loss of antinociceptive efficacy. Similarly, the initially robust efficacy of morphine in rats with SCI diminished over time with repeated treatment. The data suggest that sustained efficacy with a CB receptor agonist could depend on the pain state or the pain symptom and may be amenable to long-term therapeutic usage.

An increased understanding of the mechanism of pain has lead to the identification of several potential molecular targets that could lead to the development of efficacious analgesic drugs [24]. However, considerable time will be required to develop compounds that are selective for these targets into drugs with little or no adverse side effects. Alternatively, sources of analgesic drugs, such as marijuana, can be found in nature and have been used since ancient times [25].

THC, an active component of marijuana, has been demonstrated to have antinociceptive effects in animal pain models [26]. The antinociceptive effect of THC is significantly decreased with intracerebroventicular pre-treatment with the CB_1 receptor antagonist SR 141716A, demonstrating that the effect of THC is mediated via brain CB_1 receptors [27]. These receptors are found in various central nervous system areas that are involved in pain perception and

modulation, such as the periaqueductal grey, the thalamus, and the spinal cord dorsal horn [28]. THC is analgesic in some, but not all, types of clinical pains [17,29–32]. Although THC is available in capsule form (dronabinol), inhalation, rather than oral administration, appears to be both titratable and a more rapid means of delivering THC to the bloodstream [29,33]. A recent study demonstrated the analgesic efficacy of an oral-mucosal aerosolized formulation of a THC mixture in patients with central and peripheral neuropathic pain [17]. Formulation issues will need to be resolved if natural cannabinoids such as THC are to be used clinically.

Several synthetic cannabinoids, such as WIN, are potent agonists to the CB_1 receptor and have demonstrated antinociceptive effects in preclinical pain models. Whether these molecules have been tested in humans is unknown, so the clinical utility and safety of these molecules are unknown. An advantage, however, of synthetic molecules over marijuana is that a single molecule, rather than the mixture of characterized and uncharacterized ligands found within marijuana, may be accurately administered for clinical use. Also, further development of a molecule with defined characteristics may improve its chemical properties over those of, for example, THC. Such improvements include better water solubility, metabolic stability, and affinity to a particular CB receptor subtype [34]. The receptor binding affinity (K_i) for THC is 41 nM and 36 nM to the human CB_1 and CB_2 receptors, respectively [35]. By contrast, WIN is much more potent, with K_i = 2 nM and 0.3 nM, and has no binding activity at receptors related to pain modulation (e.g., opiate receptors) [36]. The antinociceptive duration of WIN, possibly due to its chemical structure, is as long as 3 to 4 h following subcutaneous injection, whereas the duration of THC, up to 1.5 h, is much shorter [37–38]. For clinical use, a synthetic CB ligand with known properties clearly offers pharmacological properties superior to those of a natural CB ligand.

The current study demonstrated a consistent antinociceptive effect of WIN dosed over a 7-day period in rats with neuropathic SCI pain. The intermediate and high doses of WIN showed sustained efficacy for 7 days; the A50 dose at day 7 did not significantly differ from the A50 dose at day 1. By contrast, the efficacy of WIN gradually diminished over a 5-day treatment period in uninjured rats. The data suggest that the difference in response to a CB receptor agonist over time may depend on the pain state of the animals. In rats with chronic peripheral neuropathic pain, CB receptor agonists exhibit robust efficacy despite repeated administration [39–40]. The limited clinical data suggest that chronic pain patients do not develop analgesic tolerance [30]. Following a peripheral nerve injury, expression of CB_1 receptors increases in the ipsilateral dorsal root ganglia, spinal cord dorsal horn, and contralateral thalamus [41–43]. Currently not known is whether such an increase in CB_1 receptors occurs after SCI or other chronic pain states, but an increase in CB_1 receptors in neural areas involved in nociception may underlie the sustained response observed in the current study.

Tolerance to the pharmacological effects of CB receptor agonists has been documented in uninjured animals [20]. In normal (nonchronic pain) subjects, tolerance to the psychological effects of marijuana has been reported, but a similar phenomenon to the analgesic effects has not been widely investigated [44]. Thus, whether analgesic tolerance develops with acute noxious stimuli such as heat, pressure, or cold is unknown. Further complicating the issue is that the relevance of these acute stimuli to clinical chronic pain is unknown [31,45]. De Vry et al. noted that behaviors that were most sensitive to a CB receptor agonist were least likely to develop tolerance (e.g., drug discrimination), whereas behaviors that were less sensitive to the agonist (e.g., hot plate test) were more likely to develop tolerance [20]. Thus, tolerance is more likely to develop if assessed with acute noxious stimuli. Verification of this hypothesis would include measuring receptor function or expression from tissues of animals treated with a CB receptor agonist over a period of time. Further studies on the influence of particular sensory stimuli may determine whether a particular type of neuropathic pain symptom is more or less sensitive to CB receptor agonists.

WIN is potent to the CB_2 receptor as well as to the CB_1 receptor. The long-term efficacy of WIN in the current study may also be mediated via the CB_2 receptor. An increase in CB_2 receptors occurs in the ipsilateral dorsal horn after nerve injury, but whether this receptor is also upregulated in the SCI state is unknown [46]. However, in other neuropathic pain models as well as the current model, most, if not all, of the efficacy of WIN appears to occur via the CB_1 receptor, because pretreatment with a CB_2 receptor antagonist did not alter antinociception [15,47–48].

The current data and the study by Yu et al. [49] suggest that clinical neuropathic SCI pain will be initially responsive to morphine, but dosing may need to be titrated to sustain efficacy [50]. Rats intrathecally dosed morphine in a different neuropathic SCI pain model developed tolerance beginning on the third day of treatment, indicating that a possible mechanism underlying morphine tolerance is a decrease of opiate receptors in the spinal dorsal horn [49]. The neural mechanism underlying CB and opiate tolerance may be similar, involving changes in receptor function and intracellular signaling over time [51–52]. Since the adverse side effects of morphine, including respiratory depression and constipation, may impose serious complications in SCI patients, alternate therapies without these side-effects may be more desirable.

CONCLUSIONS

The current study confirmed the antinociceptive effect of the nonselective CB receptor agonist WIN in two different pain models. Although the initial potencies were comparable in both models, after repeated treatment, efficacy was significantly diminished in the hot plate test. By contrast, the antinociceptive effect in SCI rats was maintained for the duration of the experimental period. Thus, the data suggest that depending on the pain state (or type of pain), a CB may have either persistent or short-term analgesic effects. SCI patient suffering may be further ameliorated by other effects of CBs, such as improving sleep quality and decreasing spasticity and anxiety [30]. Interestingly, SCI patients who used marijuana rated the obtained pain relief much higher than that of a separate group that used opiates (and yet another group that used gabapentin) [53]. Several small clinical trials in central neuropathic pain states, such as multiple sclerosis, demonstrated significant pain relief with an oromucosal spray formulation of THC and other CBs. More extensive, controlled clinical trials are needed to confirm the use of CBs in human neuropathic SCI pain.

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Abbreviations

A50
: 50 percent antinociceptive (dose)

BID
: twice daily

ANOVA
: analysis of variance

CB
: cannabinoid

***K*i**

receptor binding affinity

MPE

maximum possible effect

SCI

spinal cord injury

SEM

standard error of the mean

THC

Δ^9-Tetrahydrocannabinol

WIN

WIN 55,212-2 ((R)(+)-[2,3-Dihydro-5-methyl-3[(4-morpholinyl)methyl] pyrrolo [1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone)

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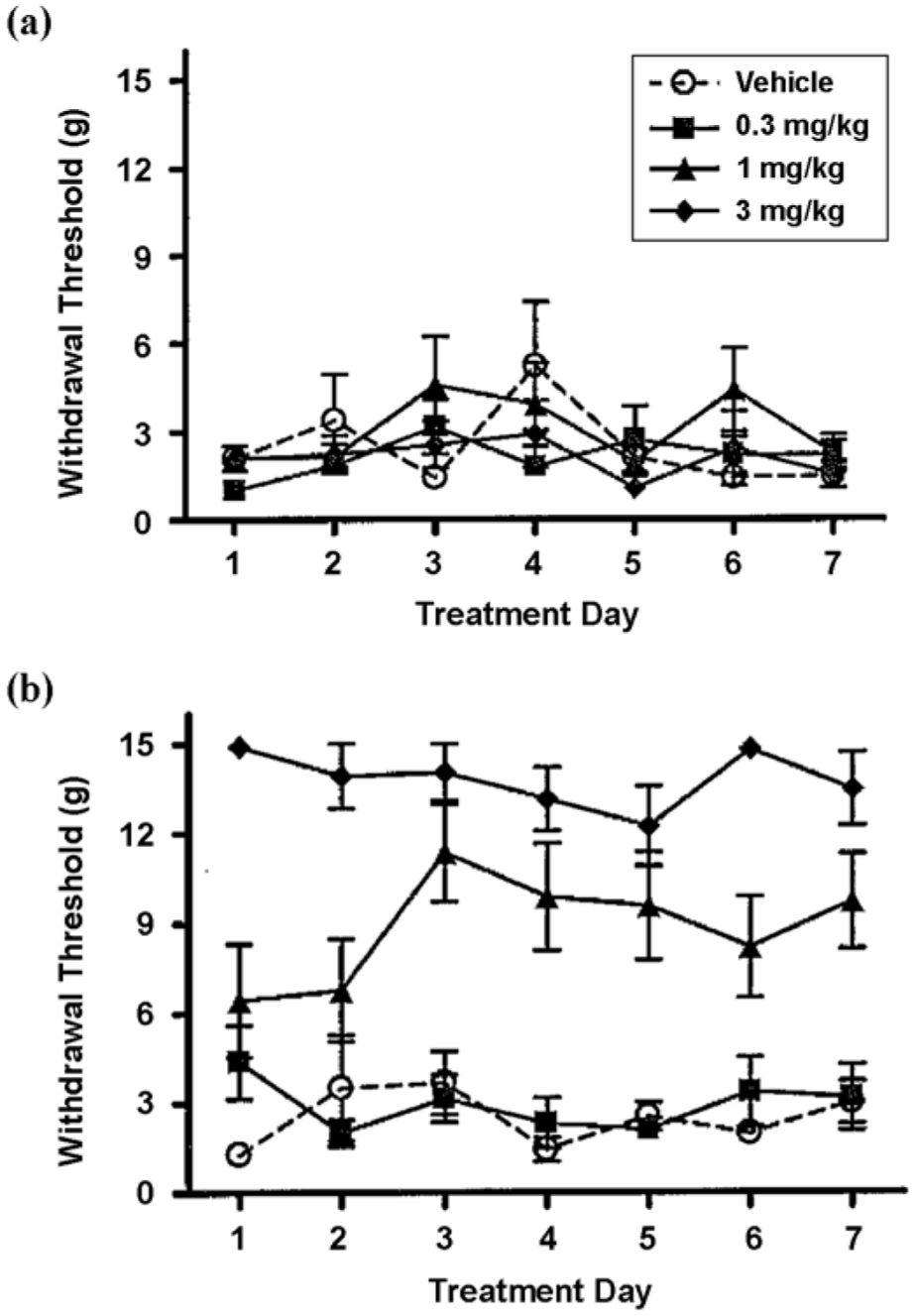

Figure 1.

Sustained antinociceptive effect of WIN 55,212-2 (WIN) over time in rats with spinal cord injury (SCI) (*n* = 5–6/group). Hindpaw withdrawal thresholds (in grams) in SCI rats were measured **(a)** before and **(b)** 30 min after injection of either WIN (0.3, 1, or 3 mg/kg) or vehicle. Rats were injected twice a day but tested only after first daily injection. Data are shown as mean ± standard error of the mean.

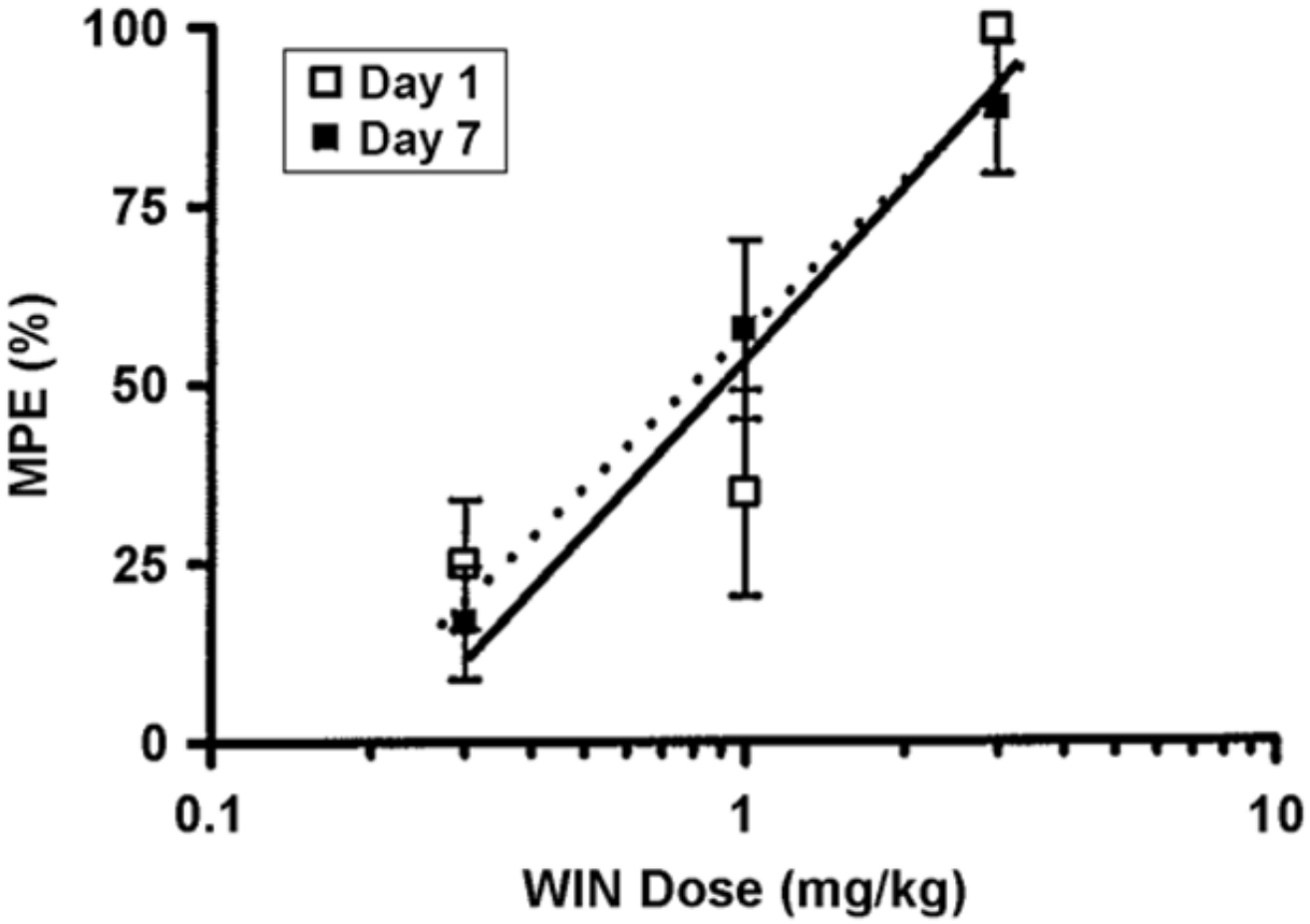

Figure 2.
Dose-response to WIN 55,212-2 (WIN) 30 min after injection in rats with spinal cord injury (*n* = 5–6 rats/group). Vertical axis is percent maximum possible effect (MPE) and horizontal axis is dose of WIN. On day 1, 50% antinociceptive (A50) dose of WIN was 0.9 mg/kg; on day 7, A50 dose was 0.8 mg/kg. Data are shown as mean ± standard error of the mean.

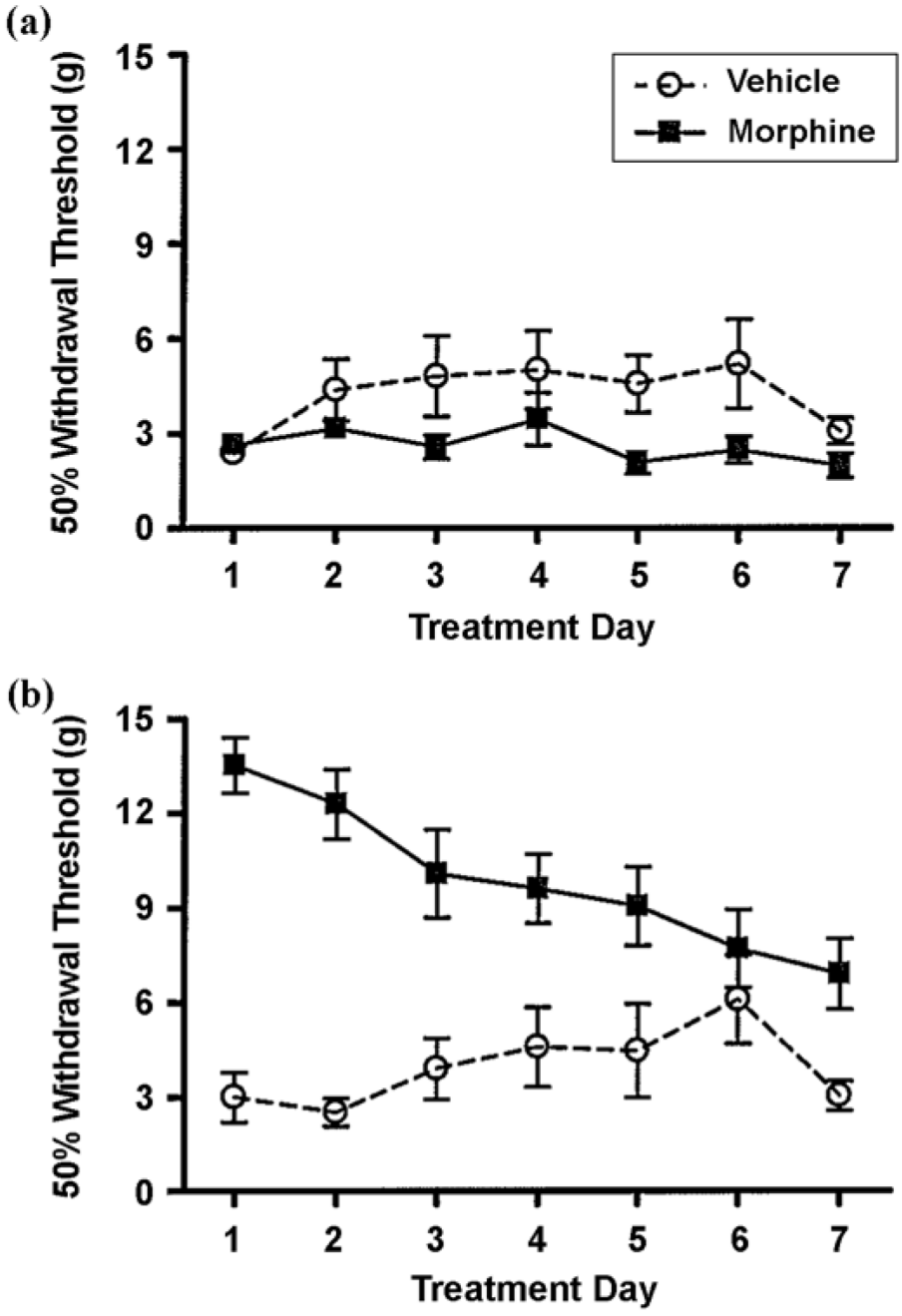

Figure 3.

Diminished antinociceptive efficacy of morphine over time in rats with spinal cord injury (SCI) (*n* = 8/group). Hindpaw withdrawal thresholds (in grams) in SCI rats were measured **(a)** before and **(b)** 30 min after injection of either morphine (3 mg/kg) or vehicle. Rats were injected twice a day but tested only after first daily injection. Data are shown as mean ± standard error of the mean.

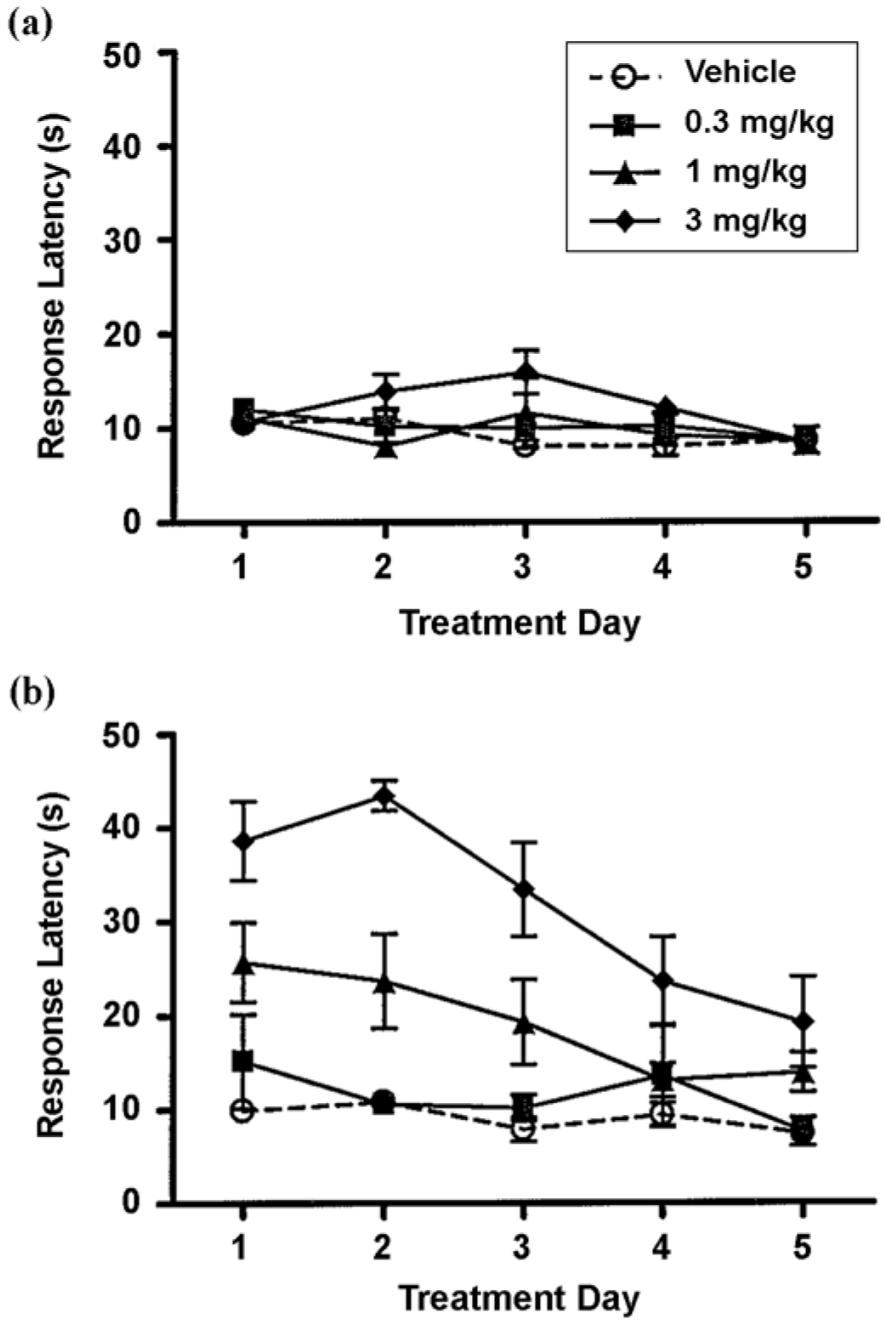

Figure 4.

Diminished antinociceptive efficacy of WIN 55,212-2 (WIN) over time in uninjured rats (*n* = 7/group). Response latencies (in seconds) of hindpaw to noxious heat stimulus were measured in uninjured rats **(a)** before and **(b)** 30 min after injection of either WIN (0.3, 1, or 3 mg/kg) or vehicle. Rats were injected twice a day but tested only after first daily injection. Data are shown as mean ± standard error of the mean.

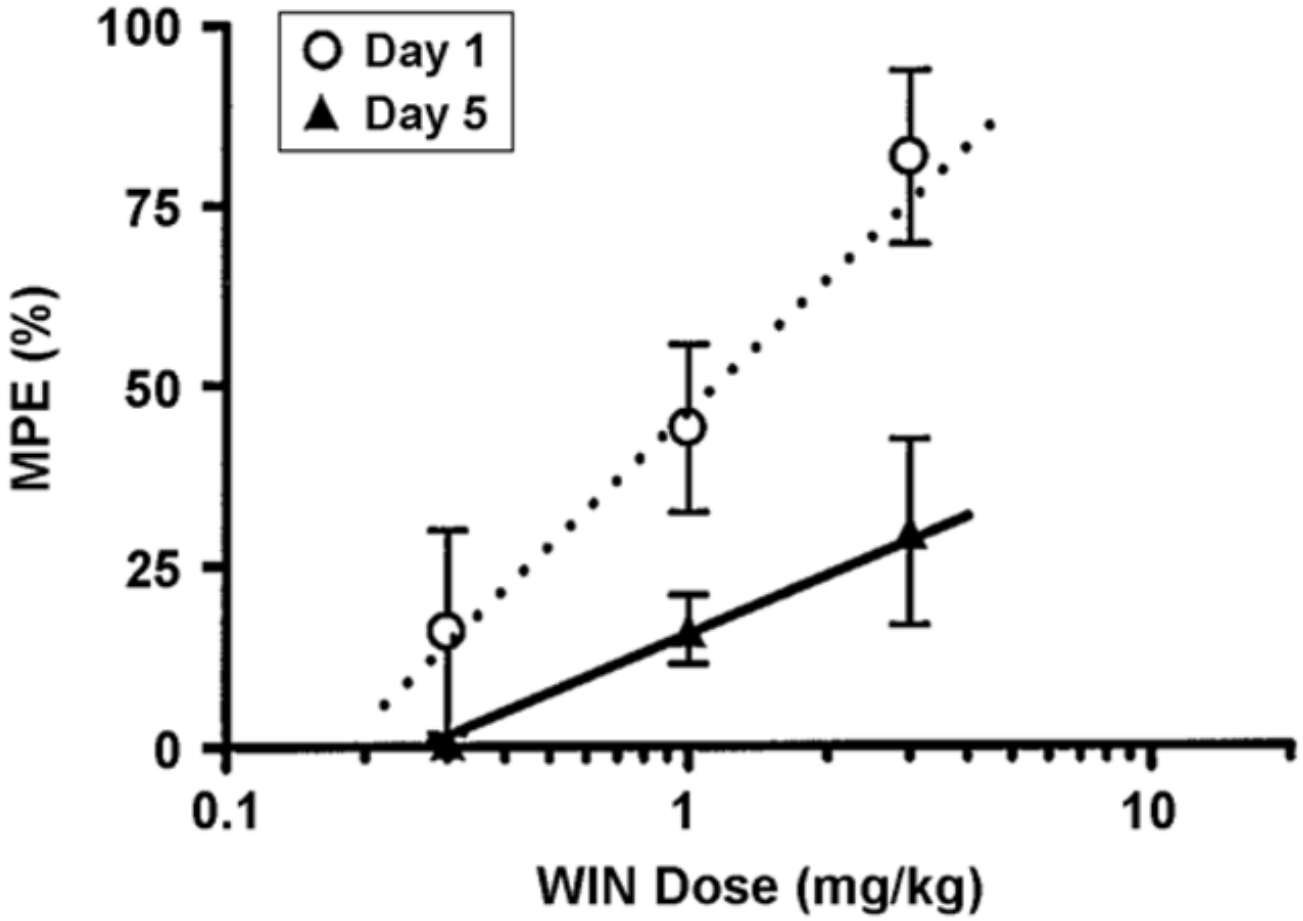

Figure 5.
Dose-response to WIN 55,212-2 (WIN) 30 min after injection in uninjured rats (*n* = 7/group). Vertical axis is percent maximum possible effect (MPE) and horizontal axis is dose of WIN. On day 1, 50% percent antinociceptive (A50) dose of WIN was 1.1 mg/kg; on day 5, A50 dose was 15.1 mg/kg. Data are shown as mean ± standard error of the mean.