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Effects of repeated dosing with mechanistically distinct antinociceptive ligands in a rat model of neuropathic spinal cord injury pain

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Abstract

A lack of efficacy of some analgesic drugs has been previously described in rats with neuropathic spinal cord injury (SCI) pain. It has been suggested that repeated dosing in these animals over time may eventually lead to efficacy. However, it is also possible that efficacy may diminish over time with repeated dosing. This study evaluated the efficacy of various drugs upon repeated dosing over time in a rat model of SCI pain. Four weeks following an acute spinal cord compression at the mid-thoracic level, rats developed decreased hind paw withdrawal threshold, suggestive of below level neuropathic hypersensitivity. Either cannabinoid (CB) receptor agonist CP 55,940, the anticonvulsant carbamazepine or gabapentin, the antidepressant amitriptyline or vehicle was administered over a period of 7 days. Neither carbamazepine nor amitriptyline demonstrated efficacy either after a single or repeated dosing. Beginning with a 50% efficacious dose of gabapentin, the effect of gabapentin in SCI rats neither increased nor decreased over the treatment period. The antinociceptive effect of CP 55,940 was maintained for the entire treatment period, which was mediated by CB1 but not CB2 receptors. The current data suggest that sustained antinociception can be obtained with some drugs in rats with neuropathic SCI pain. Furthermore, the current data do not substantiate the notion that repeated treatment with initially ineffective drugs will eventually lead to efficacy; treatments that are not acutely effective are unlikely to demonstrate clinical efficacy.

Abbreviations

ANOVA, analysis of variance; A_{50}, dose producing a 50% antinociceptive effect; 95% CL, 95% Confidence Level; b.i.d., twice daily treatment; CB, cannabinoid; CP 55,940, (-)-CP 55,940 (5-(1,1-dimethylheptyl)-2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol); Δ^9-THC, Δ^9-tetrahydrocannabinol; MPE, maximum possible effect; SCI, spinal cord injury; s.c., subcutaneous; SR 144528, 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1S, 2S,4R)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1H-pyrazole-3- carboxamide.

Introduction

There are two distinct clinical phenomena associated with repeated drug treatment over time. The first is tolerance, a decrease in efficacy at a given dose over time. Tolerance can be visualized via a rightward shift of the dose-response curve – increased doses are required to attain the same level of efficacy over time (Blumenthal and Garrison 2010). The phenomenon of tolerance can be observed in opioids, which are effective in treating acute and chronic pain. A number of preclinical studies have demonstrated tolerance to the antinociceptive effects of opioids with repeated treatments (Yu et al. 1997; Mayer et al. 1999). Several cellular mechanisms, such as changes in intracellular signaling and receptor expression due to chronic exposure to agonist, have been proposed to

underlie the decrement of opioid efficacy (Blumenthal and Garrison 2010).

The second phenomenon that may occur with repeated drug treatment is sensitization ("reverse tolerance"), the increased efficacy to the same dose of drug over time (Blumenthal and Garrison 2010). Sensitization has been suggested to be a model of drug dependency. For example, a number of drugs of abuse, including opioids, increase locomotor activity upon repeated administration over time in animals (Tassin 2008). Interestingly, a possible antinociceptive "sensitization" to drugs could occur over time, in that initially non-efficacious doses of drugs gradually become efficacious. Initially ineffective doses of gabapentin have been shown to be efficacious following repeated dosing in animal models (Hao et al. 2000; Patel et al. 2001). By contrast, clinically significant pain relief has been obtained within hours following a single dose of gabapentin (Berry and Petersen 2005). The mechanism and clinical significance of antinociceptive sensitization in preclinical animal models are not entirely clear.

A particularly challenging chronic pain population is neuropathic spinal cord injury (SCI) pain. In addition to significant impairment of motor, visceral, and autonomic functions, SCI patients experience moderate to severe pain, including below the level of the spinal injury (Widerstrom-Noga et al. 2009). Although opioids have demonstrated some efficacy on SCI pain, this class of drug may not be entirely suitable for patients with SCI due to their adverse side effects, such as respiratory depression and constipation. Improved pain management regimens need to be identified for SCI patients, such that they are able to participate in rehabilitation and social integration, thereby enhancing their quality of life.

Previous findings in our laboratory have shown that potent antinociceptive effects of a synthetic cannabinoid (CB) receptor agonist in a SCI pain model are retained even after repeated dosing, in contrast to tolerance development following repeated morphine administration (Hama and Sagen 2009). Furthermore, the same dosing regimen with a CB receptor agonist produced tolerance to antinociceptive effects in non-injured animals. The antidepressant drug amitriptyline and anticonvulsant drug carbamazepine show varying levels of efficacy after either a single dose or repeated dosing in some but not all animal models of chronic neuropathic pain (Field et al. 1999; Idanpaan-Heikkila and Guilbaud 1999; Yasuda et al. 1999; Hama and Sagen 2007b). The clinical data, however, indicate that both of these drugs are effective for neuropathic pain (McQuay et al. 1997).

Previous findings in our laboratory have also revealed an absence of efficacy following single dosing with carbamazepine and amitriptyline in an SCI pain model (Hama and Sagen 2007b). Gabapentin, which reduced neuropathic pain symptoms in this model, may show increased efficacy with repeated dosing, as demonstrated in preclinical studies. Thus, this study evaluated repeated dosing of clinically used drugs and a potent non-subtype selective analogue of Δ^9-tetrahydrocannabinol (Δ^9-THC), (-)-CP 55,940 (5-(1,1-dimethylheptyl)-2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol) (CP 55,940), in a rat model of below level neuropathic SCI pain in order to uncover a potential increase in efficacy or limited tolerance development. This study found a lack of enhanced efficacy with clinically used analgesic drugs whereas a lack of tolerance was observed with repeated treatment with a CB receptor agonist.

Materials and Methods

Rat model of neuropathic SCI pain

Male Sprague–Dawley rats (100–130 g on arrival) were obtained from Harlan Laboratories (Indianapolis, IN). Rats were housed two per cage and allowed 5–7 days after arrival to acclimate to the animal facility. Rats were fed standard rat chow (PMI, St. Louis, MO) and water ad libitum and housed on a 12 h. dark/light cycle. Procedures were reviewed and approved by the University of Miami Animal Care and Use Committee.

A SCI was performed as previously described (Hama and Sagen 2007b). In brief, aseptic surgical technique was used and rats were anesthetized with isoflurane in O_2. Following a laminectomy to expose mid-thoracic spinal segments T6-T7, a micro-vascular clip (Harvard Apparatus, Holliston, MA) was placed vertically around the exposed spinal cord. The clip was left in the vertical position for 60 sec and then removed. The muscles were sutured closed and the skin was closed with wound clips. Following surgery, rats were returned to their home cages and observed for recovery from anesthesia. Bladder function was observed 1–2 days following SCI surgery.

Three separate groups of SCI rats were used and rats were tested once (see below). Following behavioral testing at the end of the study, rats were humanely euthanized by CO_2 overdose.

Measurement of below level mechanical hypersensitivity

Four weeks after SCI, baseline withdrawal threshold of the hind paws to a non-noxious mechanical stimulus was measured with nylon von Frey filaments (Stoelting, Wood Dale, IL). The up-down method was used, utilizing the same set of filaments as previously described (Chaplan et al. 1994). The series of responses to the filaments were recorded and converted to a 50% withdrawal threshold

(in g). To be included in the study, SCI rats needed a withdrawal threshold of 4 g or less. During the study, drug-treated SCI rats that did not respond to the highest force filament were assigned a withdrawal threshold of 15 g.

Drug testing in SCI rats

There were three separate groups of SCI rats that were tested for hind paw sensitivity to mechanical stimulation.

In the First group of SCI rats, the effect of an acute dose of CP 55,940 was evaluated over a 2-h period. Following baseline threshold determination, rats were injected with either CP 55,940 (0.01–0.3 mg/kg) or an equivalent volume of vehicle and tested once every 30 min up to 120 min post injection. Since maximum efficacy was observed with 0.3 mg/kg, this dose was used in the 7-day treatment study (Second group, below) to evaluate potential tolerance development.

In the Second group, the effects of repeated drug treatment were evaluated over a 7-day period. Spinal cord-injured rats were randomized to treatment groups after baseline threshold determination. Rats were injected with either drug (CP 55,940, amitriptyline, gabapentin or carbamazepine, twice daily treatment (b.i.d.) except amitriptyline; see Table 1 for doses and dosing schedule) or an equivalent volume of the appropriate vehicle and tested up to 90 min post injection. For b.i.d. dosing, rats were injected at ~8:00 AM and 5:00 PM. Rats were tested only following the first daily injection.

In the Third group, the effects of pretreatment with CB receptor antagonists on the antinociceptive effect of CP 55,940 in SCI rats were determined. Spinal cord-injured rats were randomized to treatment groups after baseline threshold determination. Rats were pretreated with either the CB1 receptor antagonist rimonabant, CB2 selective receptor antagonist 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1S, 2S,4R)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1H-pyrazole-3-carboxamide (SR 144528) or an equivalent volume of vehicle (see Table 1 for doses). Thirty minutes after the first injection, rats were injected with either CP 55,940 (0.3 mg/kg) or an equivalent volume of vehicle. Rats were tested 30 and 60 min after the second injection.

Drugs

CP 55,940, rimonabant and SR144528 were obtained from Cayman Chemical Co. (Ann Arbor, MI). Gabapentin was obtained from Toronto Research Chemicals (Toronto, Canada) and amitriptyline HCl and carbamazepine were obtained from Sigma-Aldrich, Co. (St. Louis, MO). The weight of the salt form of amitriptyline was used for dose calculation. Table 1 lists drug vehicles and injection volumes. All other reagents were obtained from Sigma-Aldrich, Co.

All drugs were injected subcutaneous (s.c.) above the rat's hind quarter. In the case of antagonist pretreatment, one flank received antagonist and the opposite flank received CP 55,940.

The doses of amitriptyline and carbamazepine were taken from studies that demonstrated efficacy in peripheral neuropathic pain models (De Vry et al. 2004c; Bomholt et al. 2005). Furthermore, the dose of amitriptyline used in this study has been shown to significantly increase rat brain concentrations of norepinephrine and serotonin (Iyengar et al. 2004). The dose of gabapentin was also selected based on a previous study – in this study, the 50% antinociceptive dose (A_{50}) was used in order to visualize either an improvement or decrement of efficacy over time (Hama and Sagen 2007b). The doses of the CB receptor antagonists were derived from the literature (Rinaldi-Carmona et al. 1996; Scott et al. 2004).

Statistical analysis

To construct dose-effect curves, behavioral data were transformed into percent maximum possible effect (MPE) at the time of peak effect.

For withdrawal threshold,

$$\text{MPE}\% = (\text{Post-Drug treatment threshold} - \text{Baseline threshold}) \div (15\,\text{g} - \text{Baseline threshold}) \times 100$$

Table 1. Vehicles, volume of injection, and doses of drugs.

	Vehicle	Injection volume, mL/kg	Dose, mg/kg	Dose per day
Amitriptyline HCl	Saline	1	30	1
Carbamazepine	30% (2-Hydroxypropyl)-β-cyclodextrin in saline	2	30	2
CP 55,940	30% (2-Hydroxypropyl)-β-cyclodextrin in saline	1	0.3	2
Gabapentin	Saline	1	25	2
SR 144528	5% DMSO/5% Tween-80 in saline	2	1	–
Rimonabant	5% DMSO/5% Tween-80 in saline	2	3	–

All drugs and vehicles were injected s.c.

For response latencies,

$$\text{MPE}\% = (\text{Post-Drug treatment latency}) - \text{Baseline latency} \div (45\,\text{sec} - \text{Baseline latency}) \times 100$$

From the linear portion of the log-dose effect curves, the A_{50} were calculated using an online program (Ossipov 2010). The 95% confidence level (95% CL) were also calculated. To identify a possible effect of treatment over time, a repeated measures two-way analysis of variance (ANOVA) was performed with Student-Newman-Keuls for post hoc comparisons. The level of statistical significance was taken at $P < 0.05$. Data are expressed as mean ± SEM.

Results

Acute antinociceptive effect of CP 55,940 in SCI rats (First group)

At the highest dose of CP 55,940 (0.3 mg/kg), rats appeared mildly sedated. However, rats were responsive to prodding and were awake when tested. Normal movement and grooming was observed at lower doses.

Prior to injection, the mean baseline withdrawal threshold of SCI rats was 2.8 ± 0.1 g. CP 55,940 significantly increased withdrawal threshold in a dose-dependent manner ($P < 0.05$ vs. vehicle; Fig. 1A). Although maximum efficacy was rapidly obtained following injection with the highest dose, peak efficacy with lower doses (0.03 and 0.1 mg/kg) was not observed until 60–90 min following injection. The A_{50} (95% CL) at 60 and 90 min post injection were 0.05 (0.03–0.07) mg/kg and 0.04 (0.03–0.05) mg/kg, respectively. Figure 1B shows the dose-response curve for CP 55,940 60 min following dosing.

The acute antinociceptive effect of CP 55,940 in SCI rats was mediated through CB1 receptors, since pretreatment with the CB1 receptor antagonist rimonabant blocked the effect of CP 55,940 (Third group; $P < 0.05$, rimonabant vs. vehicle pretreatment; Fig. 2A) whereas pretreatment with the CB2 receptor antagonist SR 144528 did not block the effect of CP 55,940 ($P > 0.05$, SR 144528 vs. vehicle pretreatment; Fig. 2B). Pretreatment with antagonists or vehicle did not have significant effects on withdrawal threshold values.

A lack of change in efficacy following repeated dosing in SCI rats (Second group)

Prior to injection, the mean baseline withdrawal threshold of SCI rats was 2.6 ± 0.3 g. The antinociceptive effect of CP 55,940 was maintained, at full efficacy, with b.i.d. dosing over a 7-day observation period (Fig. 3). Treatment with vehicle over time did not affect withdrawal threshold values in SCI rats. Daily pre-injection baseline threshold values from either CP 55,940 or vehicle groups were not significantly altered over time ($P > 0.05$ vs. day 1).

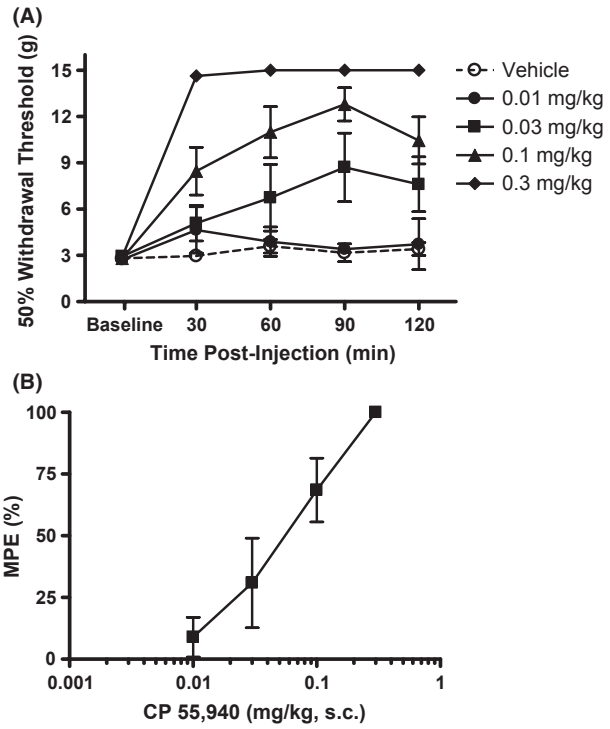

Figure 1. Antinociceptive effect of CP 55,940 on hind paw mechanical hypersensitivity in rats with a spinal cord injury. Four weeks following a spinal cord injury rats displayed decreased withdrawal threshold values (g) of the hind paws, indicating below level mechanical hypersensitivity. Rats were tested after s.c. injection of CP 55,940 or vehicle (1 mL/kg). (A) A prolonged increase in withdrawal threshold values over time was observed following CP 55,940 treatment. By contrast, vehicle treatment did not alter threshold values. (B) Log-dose response curve of CP 55,940 at 60 min post injection. The mean A_{50} dose (95% CL) was 0.05 (0.03–0.07) mg/kg. Data are expressed as mean ± SEM. $n = 6$ rats/group.

By contrast, a similar dosing scheme of CP 55,940 in uninjured rats leads to a significant decrement in the antinociceptive effect of CP 55,940 over time (Data S1). By day 4, the percent MPE of 0.3 mg/kg CP 55,940 was significantly less than that obtained at day 1 and by day 6, the percent MPE was no different from that of vehicle as well as significantly less than the MPE at day 1.

The mean baseline withdrawal threshold of SCI rats was 2.5 ± 0.2 g prior to injection of either vehicle, carbamazepine or amitriptyline. Neither vehicle nor the drugs showed antinociceptive effects with the first treatment (Fig. 4A and B). Seven days of treatment with either carbamazepine or amitriptyline did not significantly alter withdrawal threshold values ($P > 0.05$, day 1 vs. day 7; Fig. 4A and B). Drug treatments over time were not significantly different from vehicle treatment ($P > 0.05$ vs. vehicle overall).

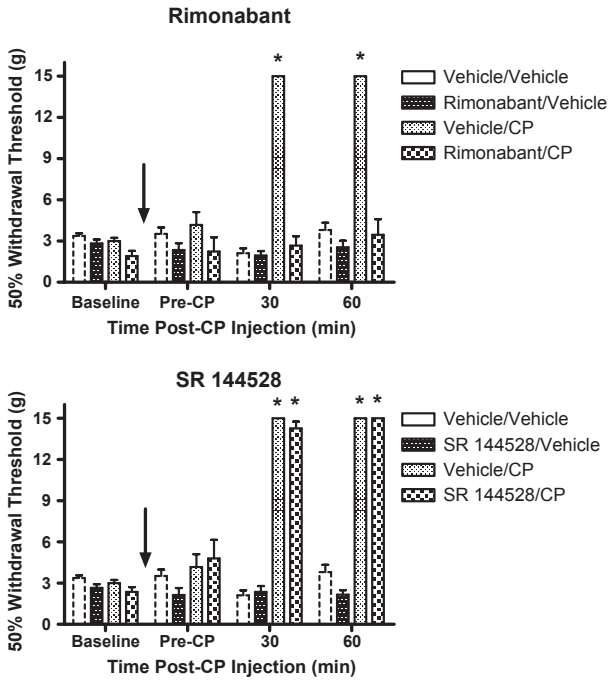

Figure 2. Effect of CB_1 receptor antagonist rimonabant (3 mg/kg, s.c.) and CB_2 receptor antagonist SR 144528 (1 mg/kg, s.c.) on the antinociceptive effect of CP 55,940 (0.3 mg/kg, s.c.) in rats with a spinal cord injury. Following baseline withdrawal threshold determination, rats were treated with CB receptor antagonist or vehicle (at "↓"). Thirty minutes later, rats were treated with either CP 55,940 or vehicle. Rats were tested 30 and 60 min following the second injection. Thus, there were four treatment groups (pretreatment /posttreatment): Vehicle/Vehicle, Vehicle/CP, antagonist/Vehicle, antagonist/CP. Pretreatment with rimonabant suppressed the antinociceptive effect of CP 55,940 (Rimonabant/CP 55,940). However, pretreatment with SR 144528 did not affect the antinociceptive effect of CP 55,940. Data are expressed as mean ± SEM. n = 6 rats/group.*$P < 0.05$ vs. Vehicle/Vehicle.

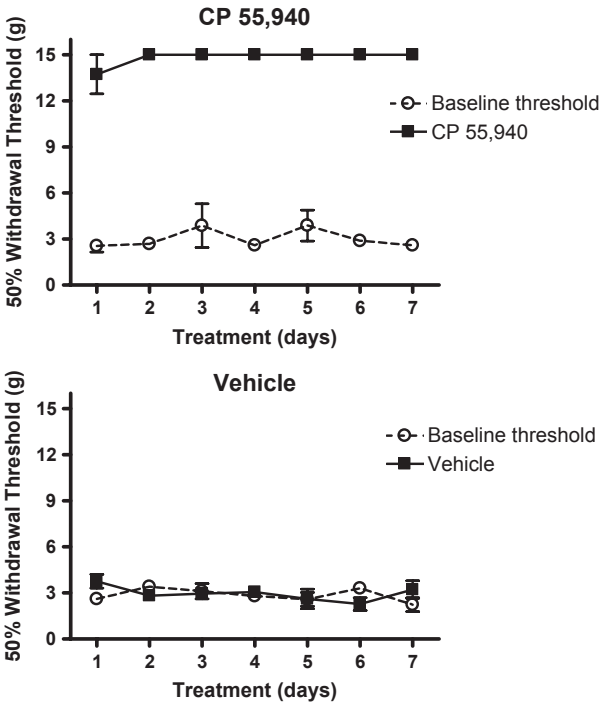

Figure 3. Effect of 7-day treatment of CP 55,940 (0.3 mg/kg, s.c., b.i.d.) in rats with a spinal cord injury. Prior to the first daily injection, baseline withdrawal threshold values were measured (open circle). Following baseline determination, rats were injected with either CP 55,940 or vehicle and rats were tested 60 min post injection (closed square). Rats were tested only following the first daily injection. The significant antinociceptive effect of CP 55,940 in spinal cord-injured rats was maintained over 7 days. By contrast, 7 days of vehicle injection did not significantly increase withdrawal threshold values. Data are expressed as mean ± SEM. n = 6 rats/group.

Prior to pretreatment with gabapentin, the mean baseline withdrawal threshold of SCI rats was 1.6 ± 0.3 g. Ninety minutes following the first injection of 25 mg/kg gabapentin on day 1, the percent MPE was 58 ± 12% ($P < 0.05$ vs. vehicle; Fig. 4C). The moderate efficacy of gabapentin was maintained over time – on day 7, the percent MPE of gabapentin was 55 ± 17%. An overall significantly antinociceptive effect of gabapentin over time was observed in comparison with vehicle ($P < 0.05$).

Treatment with vehicle over time did not significantly affect withdrawal threshold values in any of the groups ($P > 0.05$).

Discussion and Conclusion

A lack of acute antinociceptive effect in a rat model of neuropathic SCI pain was previously noted with several drugs, including amitriptyline and carbamazepine, which demonstrated efficacy in peripheral neuropathic pain models (Hama and Sagen 2007b). The lack of acute efficacy of these drugs in SCI rats could be due to a pharmacokinetic mechanism, such as an insufficient duration of drug exposure. Thus, biological activity could be observed following prolonged, rather than acute, exposure to drug. In this study, however, repeated treatments with either amitriptyline or carbamazepine did not lead to any significant antinociceptive effect in SCI rats. Similarly, repeated treatment with an initially non-efficacious dose of gabapentin has been reported to be efficacious over time (Hao et al. 2000; Patel et al. 2001). In this study, however, repeated dosing of a 50% antinociceptive dose of gabapentin did not lead to improved efficacy over time. It is unlikely that pharmacokinetics underlie the lack of efficacy of some drugs in SCI rats. More likely is that the pharmacodynamics that underlie efficacy of a given drug differ across pain models, which likely explains differential efficacy of a drug across clinical pain states.

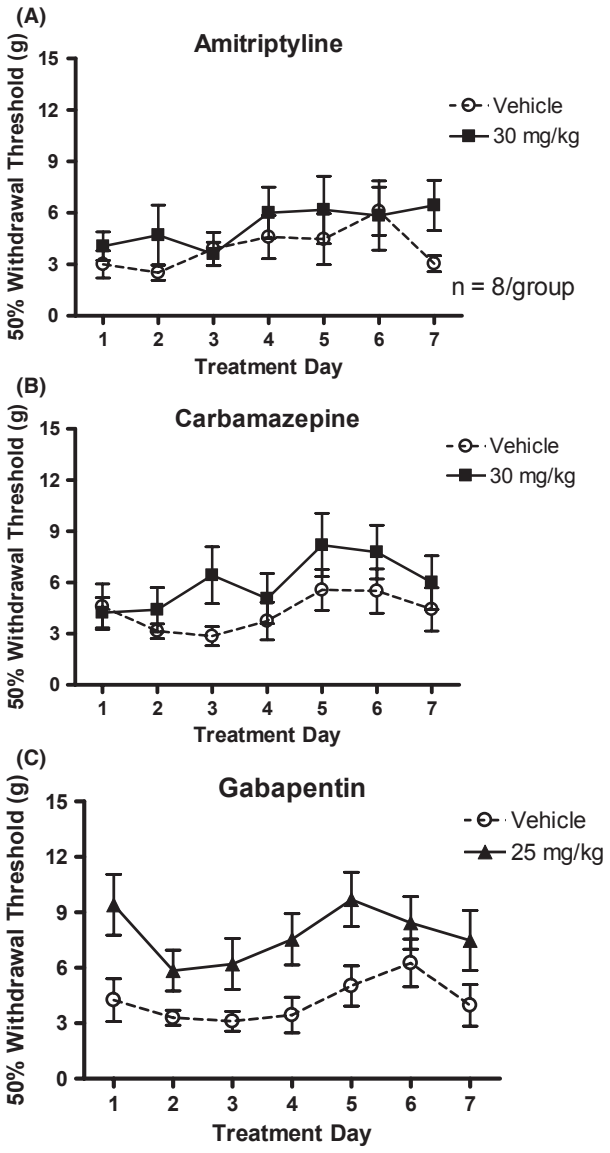

Figure 4. Repeated dosing of drugs in rats with a spinal cord injury. Rats were injected either with amitriptyline (A; 30 mg/kg, s.c., once daily), carbamazepine (B; 30 mg/kg, s.c., b.i.d.), gabapentin (C; 25 mg/kg, s.c., b.i.d.) or vehicle. Withdrawal threshold values were measured prior to the first daily injection of either drug or vehicle. Rats were tested 1 h. following injection of amitriptyline and carbamazepine and 90 min following injection of gabapentin. There was an overall lack of effect on withdrawal threshold with either amitriptyline or carbamazepine. No significant change in withdrawal threshold values were observed in vehicle-treated rats. (C) The efficacy of a 50% antinociceptive dose of gabapentin was maintained over time, neither increasing nor decreasing over time. Data are expressed as mean ± SEM. N = 8 rats/group.

One aspect of neuropathic SCI pain that makes effective treatment difficult is the interconnected and persistent neuropathological processes that mediate SCI pain. SCI leads to a number of changes in neural and glial functions, which in turn initiate and maintain persistent neural dysfunction (Yezierski 2009). Electrophysiological studies in both experimental and clinical SCI pain have documented the presence of spontaneously active spinal and supraspinal neurons, which have also been reported following peripheral tissue injury (Loeser et al. 1968; Lenz et al. 1987; Millan 1999; Yezierski 2009). While it is possible that such changes to neural function form a common basis of the symptoms reported in both peripheral and SCI neuropathic pain, given the differential efficacy of a given drug in these pain states, the basic mechanisms are likely to be distinct. Thus, etiology will be crucial factor in considering pain treatment.

It is presumed that multiple doses rather than an acute dose of a given drug are necessary to induce an antinociceptive effect, such as reported with amitriptyline in peripheral nerve-injured rats and gabapentin in rats with ischemia-induced neuropathic SCI pain (Yasuda et al. 1999; Hao et al. 2000). However, repeated dosing in this study of carbamazepine and amitriptyline did not reveal changes in efficacy. The lack of efficacy following repeated dosing of carbamazepine and amitriptyline in this study parallels clinical findings of a lack of efficacy of both drugs in SCI pain (Rintala et al. 2005). The anticonvulsant effect of carbamazepine is believed to arise from blockade of voltage-gated Na^+ channels, thereby decreasing ectopic discharges from hyperactive neurons (Kuo et al. 1997). Other drug effects could include reduced release of glutamate and other excitatory neurotransmitters from central terminals of primary afferents and from excitatory neurons as well, which, in total reduces activity of excitatory neurons throughout the CNS. Following SCI there is a large increase in glutamate and other excitatory neurotransmitters at the site of injury, which leads to excitotoxicity and the death of cells and a possible loss of central Na^+ channel function, which could explain the lack of robust efficacy of Na^+ channel blocking drugs (Yezierski 2009). The pharmacological mechanism of antidepressants such as amitriptyline is to block re-uptake of serotonin and norepinephrine, thereby increasing synaptic concentrations of these neurotransmitters. Acute lumbar intrathecal injection of serotonin and norepinephrine is antinociceptive. The lack of efficacy of amitriptyline on below level neuropathic pain suggests a significant depletion of spinal monoamines below the level of the SCI. The lack of efficacy observed acutely and with repeated dosing suggests the loss of putative targets following SCI. Perhaps acute "test" dosing of potential analgesics prior to long-term treatment could be useful in establishing a therapeutic regimen.

With respect to gabapentin, no change in efficacy, either an enhancement or decrement, was observed in the

current SCI pain model. The fact that efficacy is observed following an acute injection of gabapentin in numerous rat chronic pain models implies that the target, the α2δ subunit of the voltage-gated Ca^{2+} channel, is ubiquitous across these models (Luo et al. 2002). An upregulation of α2δ in dorsal root ganglion neurons and spinal dorsal horn, presumably within primary afferent central terminals, has been observed following peripheral nerve injury and SCI which parallels the onset of cutaneous hypersensitivity (Luo et al. 2001; Boroujerdi et al. 2011). A decrease in the expression of this subunit paralleled the remission of hind paw mechanical allodynia following peripheral nerve injury and SCI (Boroujerdi et al. 2011). The initial lack of gabapentin efficacy in some neuropathic pain models could have been due to low expression of the α2δ subunit, which over time increased, thereby enhancing gabapentin efficacy; the levels of α2δ subunit in the models that showed efficacy with repeated dosing have yet to be determined (Hao et al. 2000; Patel et al. 2001). While gabapentin and pregabalin have generally demonstrated efficacy in clinical neuropathic SCI pain, differential efficacy among SCI patients could be due to differential expression levels of the α2δ subunit. It is speculated that a stable expression pattern of α2δ over time is the basis of efficacy following an acute dose of gabapentin in the SCI state. Changes in α2δ expression over time following SCI could lead to changes in gabapentin efficacy.

Studies in preclinical pain models confirm a significant role of the CB receptor in pain modulation. In preclinical models of acute and chronic pain, the robust antinociceptive effects of natural CBs, such as Δ^9-THC, and synthetic analogues are attenuated with CB receptor-selective antagonists (Lichtman and Martin 1997; Fox et al. 2001; Hama and Sagen 2007a). The non-subtype selective CB receptor agonist CP 55,940 is potent on CB receptors in vitro but the in vivo effect is mediated through the CB1 and not the CB2 receptor (Sain et al. 2009). The current data support the findings in CB receptor knockout mice, in that the efficacy of CP 55,940 is mediated through the CB1 and not the CB2 receptor. The results of this study also confirm a previous finding using another, chemically distinct, aminoalkylindole CB receptor agonist in which antinociceptive efficacy for SCI pain was limited to the CB1 receptor (Hama and Sagen 2009).

In contrast to possible increased efficacy, repeated treatment of some drugs results in the clinical problem of tolerance, which leads to dose escalation in order to attain a consistent level of efficacy over time. A previous study showed decreased efficacy over time with repeated morphine treatment in SCI rats (Hama and Sagen 2009). By contrast, repeated treatment with CP 55,940 in this study did not lead to tolerance. Antinociceptive tolerance to CB receptor agonists, however, can be observed under specific conditions (Data S1). In uninjured rats tested in the hot plate test, significant antinociceptive tolerance to CP 55,940 is observed within 6 days of treatment (De Vry et al. 2004b). The issue of tolerance could in part be due to the model as well as due to the pharmacological properties of the drug (Costa et al. 2004; De Vry et al. 2004a). Stimulation type or intensity could potentially influence the development of CB receptor agonist tolerance. However, antinociceptive tolerance to a CB receptor agonist did not appear in peripheral nerve-injured rats, whether the stimulus was noxious mechanical pressure or heat (Costa et al. 2004). Clinically, analgesic tolerance to repeated dosing with CB receptor ligands has not been reported (Russo et al. 2007). Thus, the potential for tolerance to a given drug rests on a complex interaction between pain state, symptom and pharmacodynamics. Changes to CB receptor function or distribution in the "pain system" in chronic pain states will need to be identified in order to characterize a putative CB receptor agonist-mediated analgesic tolerance.

It is possible that the antinociceptive effects of CP 55,940 obtained in this study could be in part due to motor impairment – due to a cataleptic effect which is characteristic of CB receptor agonists and mediated by CB1 receptor activation (Fox et al. 2001). However, it has been previously observed in uninjured rats that the 50% impairment dose of CP 55,940, as determined in the rotarod test, was 0.27 mg/kg (s.c., 3 h post injection) and 0.52 mg/kg (s.c. 3 h post injection) in the "catalepsy test", which is about fivefold and 10-fold greater, respectively, than the A_{50} calculated in this study (0.05 mg/kg, s.c., 1 h post injection) (Fox et al. 2001). Thus, most of the effect observed in raising withdrawal threshold in SCI rats is due to an antinociceptive mechanism rather than due primarily to motoric deficits.

CB receptor agonists have demonstrated clinical efficacy in various types of pain states, including SCI pain (Cardenas and Jensen 2006; Hama and Sagen 2007a). The acute enhancement of mood, improvement in sleep and increase in appetite also obtained with CB may be of therapeutic benefit for some chronic pain patients (Russo et al. 2007). Additionally, the clinical analgesic effect of CB is rapid in onset and tolerance to the effect does not appear to develop over time. Nonetheless, whether there is appreciable clinical tolerance to CB receptor agonists as well as to opioids and its clinical significance are areas of active debate (Jones et al. 1976; Kalso et al. 2004; Russo et al. 2007; Anand et al. 2010).

On a different level, there will be a need to overcome social and political controversy surrounding the dispensing of *Cannabis sativa* for medical use in general. Nonetheless, the current data indicate that CB receptor agonists can be useful long-term treatments for

neuropathic SCI pain. The psychomimetic effects of CBs, likely mediated by CB1 receptors, may not suit all patients, so there is a need for finding effective therapeutics utilizing other mechanisms without the potential for decreasing efficacy over time.

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Disclosure

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. The acute antinociceptive effect of CP55,940 decreases with repeated treatment.