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RESEARCH PAPER

Activation of cannabinoid CB_1 and CB_2 receptors suppresses neuropathic nociception evoked by the chemotherapeutic agent vincristine in rats

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Background and purpose: The ability of cannabinoids to suppress mechanical hypersensitivity (mechanical allodynia) induced by treatment with the chemotherapeutic agent vincristine was evaluated in rats. Sites of action were subsequently identified.
Experimental approach: Mechanical hypersensitivity developed over the course of ten daily injections of vincristine relative to groups receiving saline at the same times. Effects of the CB_1/CB_2 receptor agonist WIN55,212-2, the receptor-inactive enantiomer WIN55,212-3, the CB_2-selective agonist (R,S)-AM1241, the opiate agonist morphine and vehicle on chemotherapy-induced neuropathy were evaluated. WIN55,212-2 was administered intrathecally (i.t.) or locally in the hindpaw to identify sites of action. Pharmacological specificity was established using competitive antagonists for CB_1 (SR141716) or CB_2 receptors (SR144528).
Key results: Systemic administration of WIN55,212-2, but not WIN55,212-3, suppressed vincristine-evoked mechanical allodynia. A leftward shift in the dose-response curve was observed following WIN55,212-2 relative to morphine treatment. The CB_1 (SR141716) and CB_2 (SR144528) antagonists blocked the anti-allodynic effects of WIN55,212-2. (R,S)-AM1241 suppressed vincristine-induced mechanical hypersensitivity through a CB_2 mechanism. Both cannabinoid agonists suppressed vincristine-induced mechanical hypersensitivity without inducing catalepsy. Spinal sites of action are implicated in cannabinoid modulation of chemotherapy-induced neuropathy. WIN55,212-2, but not WIN55,212-3, administered i.t. suppressed vincristine-evoked mechanical hypersensitivity at doses that were inactive following local hindpaw administration. Spinal coadministration of both the CB_1 and CB_2 antagonists blocked the anti-allodynic effects of WIN55,212-2.
Conclusions and implications: Cannabinoids suppress the maintenance of vincristine-induced mechanical allodynia through activation of CB_1 and CB_2 receptors. These anti-allodynic effects are mediated, at least in part, at the level of the spinal cord.
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Abbreviations: i.pl., intraplantar; PLSD, protected least significant difference

Introduction

Painful peripheral neuropathy is a common side-effect induced by diverse classes of chemotherapeutic agents including the vinca alkaloids (for example, vincristine), taxane-derived (for example, paclitaxel) and platinum-derived (for example, cisplatin) compounds. The choice of chemotherapeutic agent, dose schedule, type of cancer and presence of concomitant medical problems all affect the incidence and severity of chemotherapy-induced neuropathy (Sandler *et al*., 1969; Polomano and Bennett, 2001a; Bacon *et al*., 2003; Cata *et al*., 2006b).

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Vincristine has been postulated to induce anti-tumour effects through alteration of cytoskeletal structure and disorientation of microtubules (Tanner *et al*., 1998; Topp *et al*., 2000). Neurofilament accumulation in cell bodies and proximal axons may induce paraesthesiae and dysaesthesiae in the periphery where results of axonal transport disruption would initially be evident (Topp *et al*., 2000). Chemotherapy-induced neuropathy has also been observed in the absence of morphological damage to primary afferents; these latter studies demonstrate that chemotherapy-induced neuropathy is not dependent upon microtubule disruption (Polomano *et al*., 2001b). Chemotherapy-induced neuropathy may result from dysregulation of cellular calcium homoeostasis attributable to atypical mitochondrial function (Flatters and Bennett, 2006; Siau and Bennett, 2006).

Vincristine-induced neuropathy limits dosing and duration of potentially life-saving anti-cancer treatment (Jackson *et al*., 1988). Aspirin, ibuprofen and celebrex are commonly prescribed to patients to treat chemotherapy-induced neuropathy but show limited efficacy (Lynch *et al*., 2004). The absence of confirmed treatments for chemotherapy-evoked neuropathy makes the identification of effective alternative analgesics an urgent medical need.

Cannabinoids – drugs that share the same target as Δ^9-tetrahydrocannabinol, the psychoactive ingredient in cannabis – suppress neuropathic nociception in animal models of traumatic nerve injury through cannabinoid CB_1 and CB_2 receptor-specific mechanisms (Herzberg *et al*., 1997; Bridges *et al*., 2001; Fox *et al*., 2001; Ibrahim *et al*., 2003; LaBuda and Little, 2005; Sagar *et al*., 2005; Whiteside *et al*., 2007). CB_1 receptors are most prevalent in the central nervous system (CNS) (Zimmer *et al*., 1999). CB_2 receptors are expressed predominantly (Munro *et al*., 1993; Buckley *et al*., 2000), but not exclusively (Van Sickle *et al*., 2005; Beltramo *et al*., 2006), outside the CNS. CB_2 is markedly upregulated in rat spinal cord and dorsal root ganglion following spinal nerve ligation (Zhang *et al*., 2003; Wotherspoon *et al*., 2005; Beltramo *et al*., 2006), suggesting that additional neuroanatomical substrates may underlie CB_2-mediated antihyperalgesic actions in neuropathic pain states.

The mixed CB_1/CB_2 receptor agonist WIN55,212-2 suppresses paclitaxel-induced neuropathic nociception through a CB_1 mechanism (Pascual *et al*., 2005). However, mechanisms underlying development of painful peripheral neuropathies induced by diverse chemotherapeutic agents remain poorly understood (for a review see Cata *et al*., 2006b). Dissimilar neuropathic pain symptoms may be induced by different classes of chemotherapeutic agents and such syndromes, in turn, may respond differently to pharmacological treatments (Flatters and Bennett, 2004). Whether cannabinoids suppress neuropathic nociception evoked by vincristine treatment is unknown. We used the mixed CB_1/CB_2 agonist WIN55,212-2 and the CB_2-selective agonist AM1241 to investigate the contribution of both CB_1 and CB_2 receptors to cannabinoid modulation of chemotherapy-evoked painful neuropathy. We subsequently identified the site of action for cannabinoid anti-allodynic effects through site-specific injections of WIN55,212-2 at spinal and peripheral levels.

Methods

Animals

Two hundred and forty-three adult male Sprague–Dawley rats (223–402 g; Harlan, Indianapolis, IN, USA) were used in these experiments. All procedures were approved by the University of Georgia Animal Care and Use Committee and followed the guidelines for the treatment of animals of the International Association for the Study of Pain (Zimmermann, 1983). Bedding containing metabolized vincristine was treated as biohazardous waste and disposed off, according to the appropriate institutional guidelines.

General experimental methods

Drug effects were evaluated using a single stimulus modality to prevent development of behavioural sensitization to cutaneous stimulation. Baseline responses to mechanical or thermal stimulation of the hindpaw were established on day zero. Rats subsequently received daily intraperitoneal (i.p.) injections of either vincristine sulphate (0.1 ml/kg/day i.p.) or saline (1 ml/kg/day i.p.) over 12 days, immediately following behavioural testing. The treatment paradigm consisted of five daily injections, followed by a 2-day interval where no injections were administered, followed by five subsequent daily injections, as described previously (Weng *et al*., 2003). In all studies, the experimenter was blinded to the drug condition. Weights were recorded daily.

Assessment of mechanical withdrawal thresholds

Mechanical withdrawal thresholds were assessed using a digital Electrovonfrey Anesthesiometer (IITC model Alemo 2290-4; Woodland Hills, CA, USA) equipped with a rigid tip. Rats were placed underneath inverted plastic cages and positioned on an elevated mesh platform. Rats were allowed to habituate to the chamber for 10–15 min before testing. Stimulation was applied to the midplantar region of the hind paw through the floor of the mesh platform. Mechanical stimulation was terminated upon paw withdrawal; consequently, there was no upper threshold limit set for termination of a trial. Mechanical withdrawal thresholds were measured in duplicate for each paw before and 24 h following every injection of vincristine or saline. The last injection of vincristine or saline was administered on day 11. On the test day (day 12), baseline mechanical withdrawal thresholds were assessed (approximately 24 h following the last injection of vincristine or saline) and effects of pharmacological manipulations were evaluated. Nocifensive responses were observed in vincristine-treated animals at forces (*g*) that failed to elicit withdrawal responses before chemotherapy treatment. Vincristine-induced decreases in mechanical paw withdrawal thresholds (assessed with the Electrovonfrey Anesthesiometer) were therefore defined as mechanical allodynia.

Following assessment of baseline mechanical withdrawal thresholds (on day 12), vincristine-treated animals received systemic injections of WIN55,212-2 (0.75, 1.5 or 2.5 mg kg^{-1} i.p.; $n=8$ per group) or vehicle ($n=8$). Separate groups received either the receptor-inactive enantiomer WIN55,212-3 (2.5 mg kg^{-1} i.p.; $n=8$), the CB_2-selective agonist AM1241 (2.5 mg kg^{-1} i.p.; $n=8$) or the opiate agonist morphine (2.5 or 8 mg kg^{-1} i.p.; $n=8$ and 4, respectively). The low-dose of morphine was selected based upon its ability to suppress neuropathic pain behaviour in a spinal nerve ligation model (LaBuda and Little, 2005; Joshi *et al*., 2006) and to induce antinociception (Ibrahim *et al*., 2006). The dose of AM1241 employed was similar to that which normalized mechanical paw withdrawal thresholds following spinal nerve ligation (Ibrahim *et al*., 2003). To determine pharmacological specificity, groups received either WIN55,212-2 (2.5 mg kg^{-1} i.p.) coadministered with either SR141716 (2.5 mg kg^{-1} i.p.; $n=8$) or SR144528 (2.5 mg kg^{-1} i.p.; $n=8$), AM1241 (2.5 mg kg^{-1} i.p.) coadministered with

either SR141716 (2.5 mg kg^{-1} i.p.; $n=8$) or SR144528 (2.5 mg kg^{-1} i.p.; $n=8$) or either antagonist administered alone ($n=8$ per group). In all studies, mechanical withdrawal thresholds were evaluated (on day 12) approximately 24 h following the last injection of vincristine. Paw withdrawal thresholds were measured before (baseline) and at 30 and 60 minutes post-injection of drug or vehicle. To evaluate the possible resolution of vincristine-induced painful peripheral neuropathy, vincristine-treated rats receiving vehicle were additionally evaluated for the presence of mechanical allodynia 31 days following the last injection of vincristine.

Assessment of thermal paw withdrawal latencies

Paw withdrawal latencies to radiant heat were measured in duplicate for each paw using the Hargreaves test (Hargreaves *et al*., 1988) and a commercially available plantar stimulation unit (IITC model 33; Woodland Hills, CA, USA). Rats were placed underneath inverted plastic cages positioned on an elevated glass platform. Rats were allowed to habituate to the apparatus for 10–15 min before testing. Radiant heat was presented to the midplantar region of the hind paw through the floor of the glass platform. Stimulation was terminated upon paw withdrawal or after 20 s to prevent tissue damage. Thermal paw withdrawal latencies are reported as the mean of two sets of duplicate determinations averaged across paws. Thermal withdrawal latencies were evaluated before (day 0) and on days 3, 6, 9 and 12 following administration of either vincristine ($n=12$) or saline ($n=6$) as described above. The same animals were subsequently tested for the presence of mechanical allodynia (on day 12) using methods described above.

Intrathecal catheter implantation

Intrathecal catheters (PE10 tubing, Clay Adams, Parsippany, NJ, USA) were surgically implanted under pentobarbital/ketamine anaesthesia into the spinal subarachnoid space through an incision in the atlanto-occipital membrane (Yaksh and Rudy, 1976; Hohmann and Herkenham, 1998a). Catheters were implanted to a depth of 8.5 cm, secured to the skull and the distal end was heat-sealed. Animals exhibiting any signs of motor impairment (for example impairment in walking on a wire cage cover or impaired righting reflexes) induced by catheter implantation were immediately killed. Approximately 10% of animals which underwent catheter implantation showed evidence of motor impairment and consequently never received subsequent testing or vincristine or saline treatment. Animals were allowed to recover for at least 5 days following surgery before determination of baseline paw withdrawal thresholds and initiation of vincristine or saline treatment.

Site of action

An initial experiment was performed to determine if i.t. administration of the β-cyclodextrin vehicle ($n=6$) altered mechanical withdrawal thresholds relative to groups that were surgically implanted with the catheter, but did not receive an i.t. injection ($n=4$). Other vincristine-treated groups received WIN55,212-2 (10 μg or 30 μg i.t.; $n=6$ per group) or WIN55,212-3 (10 μg i.t., $n=6$). To determine pharmacological specificity of cannabinoid actions, separate groups received either WIN55,212-2 (30 μg i.t.) coadministered with either SR141716 (30 μg i.t.; $n=8$) or SR144528 (30 μg i.t.; $n=8$), WIN55,212-2 (30 μg i.t.) coadministered with both SR141716 (30 μg i.t.) and SR144528 (30 μg i.t.) concurrently ($n=6$) or either SR144528 (30 μg i.t.; $n=6$) or SR141716 (30 μg i.t.; $n=5$) administered alone. In all studies, mechanical paw withdrawal thresholds were evaluated daily as described above to verify that vincristine treatment induced mechanical allodynia relative to groups that received saline ($n=9$) at the same times. Following testing, catheter placement was verified by post-mortem injection of Fast green dye followed by dissection. No animals exhibited tissue damage due to catheter placement. In all studies, mechanical withdrawal thresholds were evaluated (on day 12) approximately 24 h following the last injection of vincristine. Paw withdrawal thresholds were measured in duplicate before (baseline) and at 5, 30 and 60 minutes post-injection of drug or vehicle.

To evaluate possible peripheral sites of cannabinoid action, WIN55,212-2 or vehicle was administered locally in the paw. Intraplantar (i.pl.) injections were performed unilaterally into the plantar surface of the hindpaw for each animal on the test day (day 12). Vincristine-treated rats received either vehicle ($n=7$) or WIN55,212-2 (30 or 150 μg; $n=9$ per group) locally in the hindpaw. Right or left paw injections were counterbalanced between subjects. Thresholds were measured in both the injected and non-injected paw for all animals before (baseline) and at 30 min post-injection.

Catalepsy testing

Catalepsy testing was performed on test day 12 using the bar test (Pertwee and Wickens, 1991; Martin *et al*., 1996) in rats previously evaluated for responsiveness to thermal stimulation. Rats were returned to their home cages for at least 30 min following assessment of thermal paw withdrawal latencies, before initiation of baseline catalepsy assessment. Animals were placed on a stainless steel bar suspended 9 cm above a flat platform; forepaws were suspended over the bar and hindpaws were in contact with the table as described previously (Martin *et al*., 1996). Catalepsy was reassessed in vincristine-treated animals receiving either vehicle ($n=6$) or WIN55,212-2 (2.5 mg kg^{-1} i.p.; $n=6$). A separate group of vincristine-treated animals (which did not undergo thermal testing) received AM1241 (2.5 mg kg^{-1} i.p.; $n=6$). Two groups of otherwise naive animals received WIN55,212-2 (2.5 or 10 mg kg^{-1} i.p.; $n=6$ per group). Time spent immobile on the bar was measured in triplicate for all groups at 30, 45 and 60 min post-drug injection.

Statistical analyses

Data were analysed using analysis of variance (ANOVA) for repeated measures, ANOVA or planned comparison unpaired *t*-tests as appropriate. The Greenhouse–Geissser correction was applied to all repeated factors. Paired *t*-tests were also used to compare post-drug thresholds with pre-vincristine

(baseline) thresholds. The percent (%) reversal of mechanical allodynia was calculated at the time point of maximal cannabinoid anti-allodynic efficacy using the formula:

$$\frac{(\text{day 12 post-injection threshold} - \text{day 12 preinjection threshold})}{(\text{day 0 previncristine baseline threshold} - \text{day 12 preinjection threshold})} \times 100$$

Post hoc comparisons were performed using Fisher's protected least significant difference (PLSD) test. $P<0.05$ was considered statistically significant.

Drugs and chemicals

Vincristine sulphate was obtained from Tocris Cookson (Ellisville, MO, USA). WIN55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate), WIN55, 212-3 (*S*(–)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]-pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate salt), morphine sulphate and *β*-cyclodextrin were purchased from Sigma Aldrich (St Louis, MO, USA). (*R,S*)-AM1241 ((*R,S*)-(2-iodo-5-nitro-phenyl)-[l-(l-methyl-piperidin-2-ylmethyl)-l*H*-indol-3-yl]-methanone) was synthesized in the laboratory of one of the authors (AM). SR141716 (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) and SR144528 (*N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide) were provided by NIDA. Vincristine sulphate was dissolved in a vehicle of 0.9% saline. All other drugs were dissolved in a vehicle of 10% ethanol, 10% emulphur and 80% saline for systemic administration and administered in a volume of 1 ml/kg bodyweight with one exception. In experiments where antagonists were co-administered with AM1241, due to limits in solubility, the total injection volume was 1.5 ml/kg. Drugs were dissolved in 45% *β*-cyclodextrin as described previously (Hohmann *et al*., 1998b) for i.t. and i.pl. administration. Drug or vehicle was administered in volumes of 10 and 50 *μ*l for i.t. and i.pl. administration, respectively.

Results

General results

Body weight did not differ between groups before administration of vincristine or saline. Normal weight gain was observed over the injection time course in saline-treated animals ($F_{1,40}=41.515$, $P<0.0002$; Figure 1a). By contrast, vincristine-treated groups showed an absence of weight gain at all post-injection intervals ($F_{11,440}=23.32$, $P<0.0002$; $P<0.001$ for each comparison; Figure 1a). Figure 1a presents changes in body weight over the course of vincristine or saline treatment for groups shown in Figure 1b. By 31 days following the last injection of vincristine, mechanical hypersensitivity had completely resolved in vincristine-treated animals receiving vehicle (i.p.) and normal weight gain was observed (data not shown).

In studies employing systemic or i.t. injections, responses to mechanical and thermal stimuli did not differ between

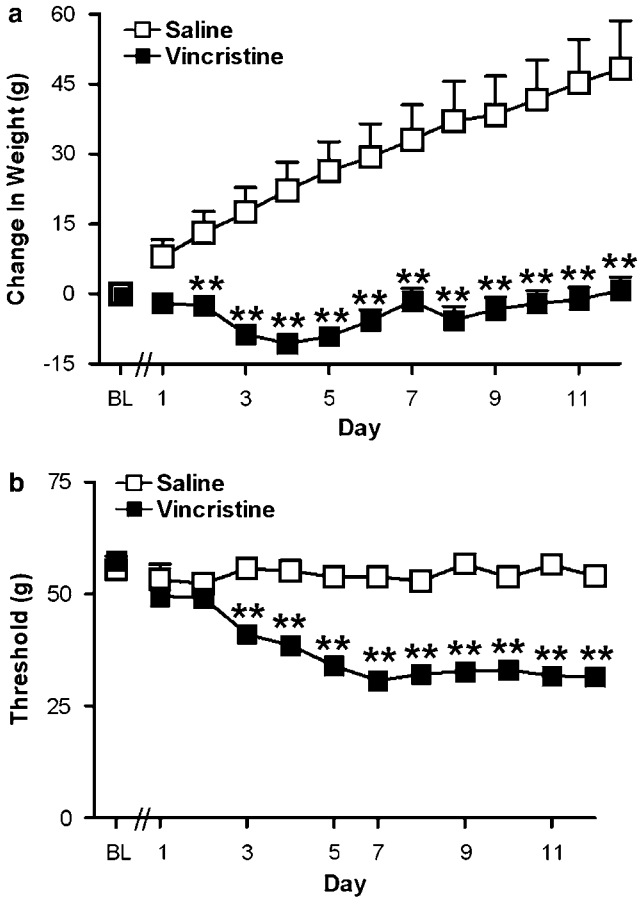

Figure 1 (**a**) Normal weight gain was absent in groups treated with the chemotherapeutic agent vincristine, relative to saline-treated controls. (**b**) Time course of vincristine-induced mechanical allodynia, as demonstrated by a lowering of the threshold for paw withdrawal to punctuate mechanical stimulation. Data are mean ±s.e.m. $^{**}P<0.001$ different from control conditions (ANOVA and Fisher's PLSD *post hoc* test).

right and left paws for any group on any given day; therefore, withdrawal thresholds are presented as the mean of duplicate measurements, averaged across paws. In studies employing unilateral i.pl. injections, results are reported for the injected and non-injected paws separately. In all studies, vincristine lowered paw withdrawal thresholds (that is equivalently in each paw) to mechanical stimulation ($P<0.0002$ for all experiments; Figures 1b, 2, 5a and 7). Modest baseline differences in paw withdrawal thresholds were observed before vincristine administration in a subset of groups ($P<0.01$ for each study; Figures 3a, c and 6a). However, on the test day, mechanical withdrawal thresholds did not differ between vincristine-treated groups before pharmacological manipulations in any study. Three animals failed to develop vincristine-induced hypersensitivity and were not used in subsequent pharmacological experiments.

Assessment of mechanical allodynia following systemic administration of WIN55,212-2

In vincristine-treated rats, WIN55,212-2 induced a dose-dependent increase in mechanical withdrawal thresholds

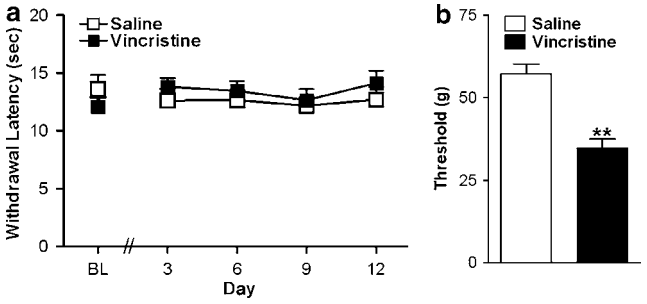

Figure 2 (**a**) Vincristine did not induce hypersensitivity to thermal stimulation relative to the control condition. (**b**) The same vincristine-treated animals showed robust mechanical allodynia (on day 12). Data are means ± s.e.m. $^{**}P<0.001$ different from control conditions (ANOVA). $N=6$–12 per group.

relative to vehicle ($F_{3,28}=5.141$, $P<0.006$, Figure 3a) and day 12 (preinjection) paw withdrawal thresholds determined before pharmacological manipulations ($F_{6,56}=6.628$, $P<0.0002$). The high dose of WIN55,212-2 (2.5 mg kg^{-1} i.p.) produced the maximal suppression of mechanical hypersensitivity and outlasted the effects of the middle (1.5 mg kg^{-1} i.p.) and low (0.75 mg kg^{-1} i.p.) doses ($P<0.02$ for all comparisons). The high dose of WIN55,212-2 effectively normalized mechanical withdrawal thresholds relative to previncristine levels (one-tailed *t*-test, $P=0.059$). WIN55,212-2 induced a dose-dependent reversal of mechanical allodynia at 30 minutes post-drug injection ($F_{3,28}=14.829$, $P<0.0002$; Figure 3b). The middle and low dose of WIN55,212-2 (0.75 and 1.5 mg kg^{-1} i.p.) produced greater than 50% reversal of mechanical allodynia ($P<.01$ for all comparisons). The high dose of WIN55,212-2 (2.5 mg kg^{-1} i.p.) produced the maximal suppression of mechanical hypersensitivity at 30 min post-injection ($P<0.002$ for all comparisons; Figure 3b).

The WIN55,212-2-induced increase in mechanical withdrawal thresholds was receptor-mediated ($F_{2,21}=17.78$, $P<0.0002$; Figure 3c); WIN55,212-2 (2.5 mg kg^{-1} i.p.) suppressed mechanical hypersensitivity relative to treatment with vehicle or the receptor-inactive enantiomer WIN55, 212-3 (2.5 mg kg^{-1} i.p.) ($P<0.0002$ for each comparison). The active but not the inactive enantiomer also increased paw withdrawal thresholds relative to day 12 preinjection thresholds ($F_{4,42}=11.236$, $P<0.0005$; Figure 3c). Mechanical withdrawal thresholds in WIN55,212-3-treated animals did not differ from vehicle at any time point.

Pharmacological specificity

In vincristine-treated rats, administration of the CB_1-selective antagonist SR141716 (2.5 mg kg^{-1} i.p.) or the CB_2-selective antagonist SR144528 (2.5 mg kg^{-1} i.p.) did not alter paw withdrawal thresholds relative to vehicle (Figure 3d). However, both antagonists blocked the suppression of vincristine-evoked mechanical allodynia induced by WIN55,212-2 ($F_{3,28}=5.79$, $P<0.004$; $P<0.05$ for each comparison; Figure 3e) and this blockade was time-dependent ($F_{6,56}=9.51$, $P<0.0002$). *Post hoc* comparisons failed to reveal a differential blockade of the anti-allodynic effects of WIN55,212-2 following treatment with either antagonist. Paw withdrawal thresholds were higher in groups receiving WIN55,212-2 alone compared to either antagonist coadministration group. Partial and complete blockade of the WIN55,212-2-induced attenuation of vincristine-induced mechanical hypersensitivity was observed at 30 and 60 min post-injection, respectively ($P<0.05$ for each comparison; Figure 3e).

WIN55,212-2 (2.5 mg/kg i.p.) produced >100% reversal of vincristine-evoked mechanical allodynia relative to vehicle treatment at 30 min post-injection ($F_{3,28}=4.009$, $P<0.02$; Figure 3f). At this time point, SR144528 ($P<0.005$, planned comparison *t*-test), but not SR141716, reliably attenuated the anti-allodynic effects of WIN55,212-2. Planned comparisons failed to reveal significant differences in reversal of vincristine-evoked mechanical allodynia observed following WIN55,212-2 coadministration with either SR144528 or SR141716 ($P>0.26$). By 60 min post-injection, both SR141716 and SR144528 produced a complete reversal of the WIN55,212-2-induced suppression of mechanical allodynia ($F_{3,28}=9.123$, $P<0.0003$; $P<.002$ for all comparisons; Figure 3f, inset).

Assessment of mechanical allodynia following systemic administration of AM1241 and morphine

WIN55,212-2 (2.5 mg kg^{-1} i.p.) and morphine (8 mg kg^{-1} i.p.) suppressed vincristine-evoked mechanical allodynia ($F_{4,31}=9.513$, $P<0.0002$; Figure 4a) relative to treatment with either vehicle, the CB_2-selective agonist AM1241 or the lower dose (2.5 mg kg^{-1} i.p.) of morphine ($P<0.01$ for each comparison). The time course of anti-allodynic effects observed was differentially affected by the experimental treatments ($F_{8,62}=3.926$, $P<0.002$). The suppression of vincristine-evoked mechanical allodynia induced by WIN55,212-2 (2.5 mg kg^{-1} i.p.) was comparable to the high dose (8 mg kg^{-1} i.p.) of morphine. By contrast, paw withdrawal thresholds in groups receiving the lower dose of morphine (2.5 mg kg^{-1} i.p.) did not differ from vehicle at any time point. A leftward shift in the dose–response curve for post-drug paw withdrawal thresholds was also observed for WIN55,212-2 relative to morphine (Figure 4b). AM1241 (2.5 mg kg^{-1} i.p.) also suppressed vincristine-evoked mechanical allodynia relative to vehicle and the low dose of morphine (2.5 mg kg^{-1} i.p.). This suppression was maximal at 30 min post-injection ($P<0.05$ for all comparisons; Figure 4a). The anti-allodynic effect of WIN55,212-2 (2.5 mg kg^{-1} i.p.) was greater ($P<0.05$) and of longer duration than that induced by AM1241 (Figure 4a). The AM1241-induced suppression of vincristine-induced mechanical hypersensitivity was similar to that induced by the low and middle doses of WIN55,212-2 (0.75 and 1.5 mg kg^{-1} i.p., respectively); thresholds were elevated at 30 min post-injection and returned to vehicle levels by 60 min post-drug ($P<0.04$ for all comparisons; Figures 4b and c).

The AM1241-induced suppression of mechanical allodynia was mediated by CB_2 receptors ($F_{2,21}=8.58$, $P<0.002$, Figure 4d). The anti-allodynic effects of AM1241 were blocked by the CB_2 antagonist SR144528 (2.5 mg kg^{-1} i.p.; $P<0.003$) but not by the CB_1 antagonist SR141716

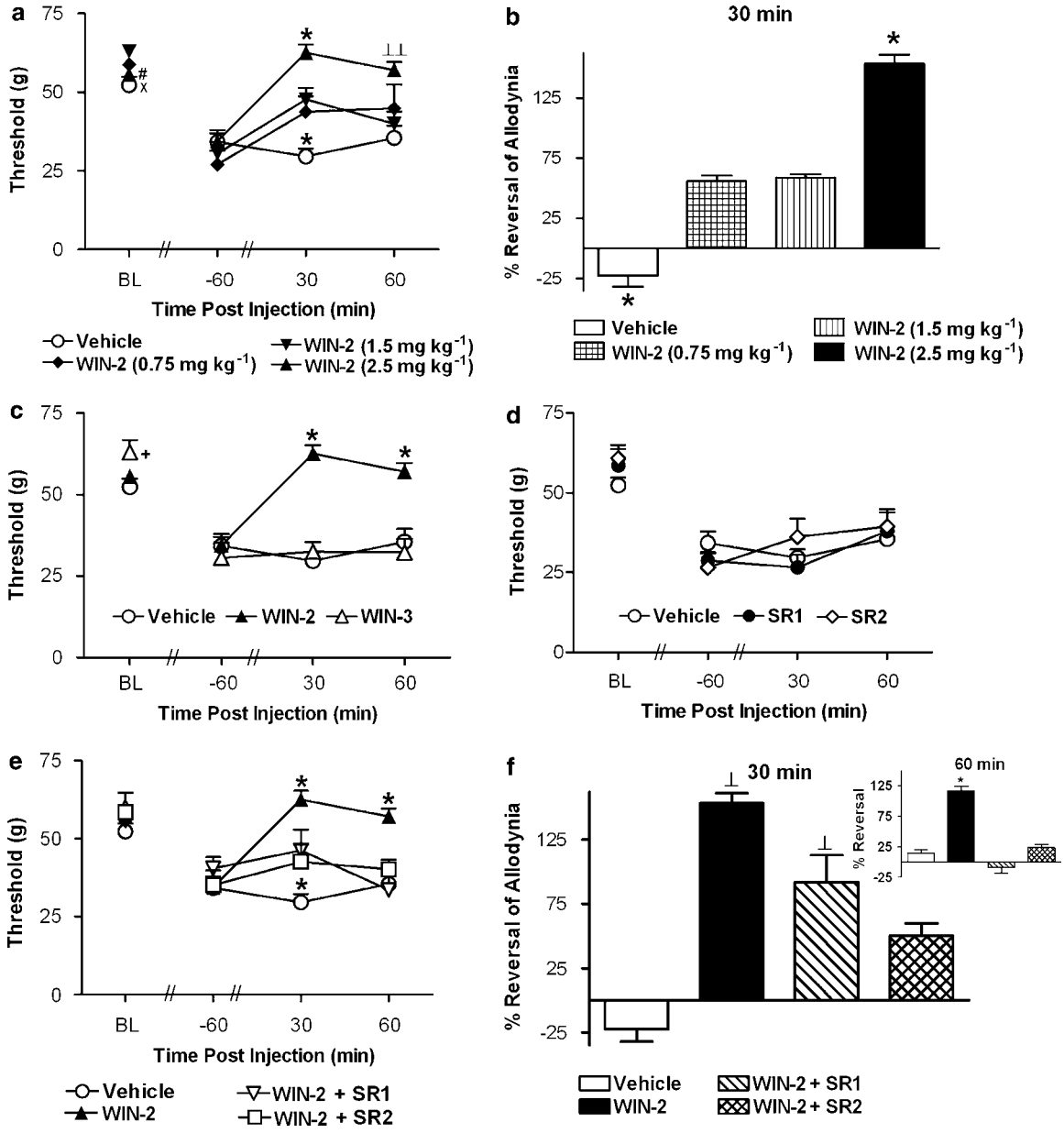

Figure 3 (**a**) The CB_1/CB_2 agonist WIN55,212-2 (WIN-2; 2.5, 1.5 and 0.75 mg kg^{-1} i.p.) induced a dose-dependent suppression of vincristine-induced mechanical allodynia, as demonstrated by an increase in the mechanical paw withdrawal threshold (on day 12). In all panels, BL denotes the baseline, day 0, paw withdrawal threshold assessed before vincristine or saline treatment. (**b**) WIN55,212-2 (2.5 mg kg^{-1} i.p.) produced a maximal reversal of mechanical allodynia at 30 min post-injection. (**c**) WIN55,212-2 (2.5 mg kg^{-1} i.p.) suppressed vincristine-evoked mechanical allodynia relative to the receptor-inactive enantiomer WIN55,212-3 (WIN-3; 2.5 mg kg^{-1} i.p.) or vehicle. (**d**) The CB_1 antagonist SR141716 (SR1; 2.5 mg kg^{-1} i.p.) and the CB_2 antagonist SR144528 (SR2; 2.5 mg kg^{-1} i.p.) did not alter vincristine-induced mechanical allodynia relative to vehicle. (**e**) Blockade of WIN55,212-2-induced anti-allodynia by SR141716 and SR144528. (**f**) Percent reversal of WIN55,212-2-induced suppression of mechanical hypersensitivity by SR141716 and SR144528 at 30 min post-injection. Inset: complete reversal of WIN55,212-2 induced anti-allodynic effects by SR141716 and SR144528 was observed at 60 min post-injection. Data are means$\pm$s.e.m. $^{*}P<0.05$ different from all groups, $^{\#}P<0.05$ different from WIN55,212-2 (1.5 mg kg^{-1} i.p.), $^{\perp\perp}P<0.01$, $^{\perp}P<0.05$ different from vehicle and WIN55,212-2 (1.5 mg kg^{-1} i.p.), $^{X}P<0.05$ different from the middle and low dose of WIN55,212-2, $^{+}P<0.05$ different from vehicle (ANOVA and Fisher's PLSD *post hoc* test). $N=8$ per group.

(2.5 mg kg^{-1} i.p.). Paw withdrawal thresholds were lower ($P<0.003$) in groups receiving AM1241 coadministered with SR144528 compared to groups receiving AM1241 in the presence or absence of SR141716 ($P<0.002$). AM1241 also increased paw withdrawal thresholds relative to day 12 preinjection thresholds ($F_{4,42}=3.087$, $P<0.03$; Figure 4d).

Assessment of thermal paw withdrawal latencies in vincristine-treated animals

Paw withdrawals latencies to thermal stimulation did not differ between vincristine and saline-treated groups at any post-injection interval (Figure 2a). Nonetheless, the same vincristine-treated group exhibited robust mechanical allodynia when compared with their saline-treated counterparts

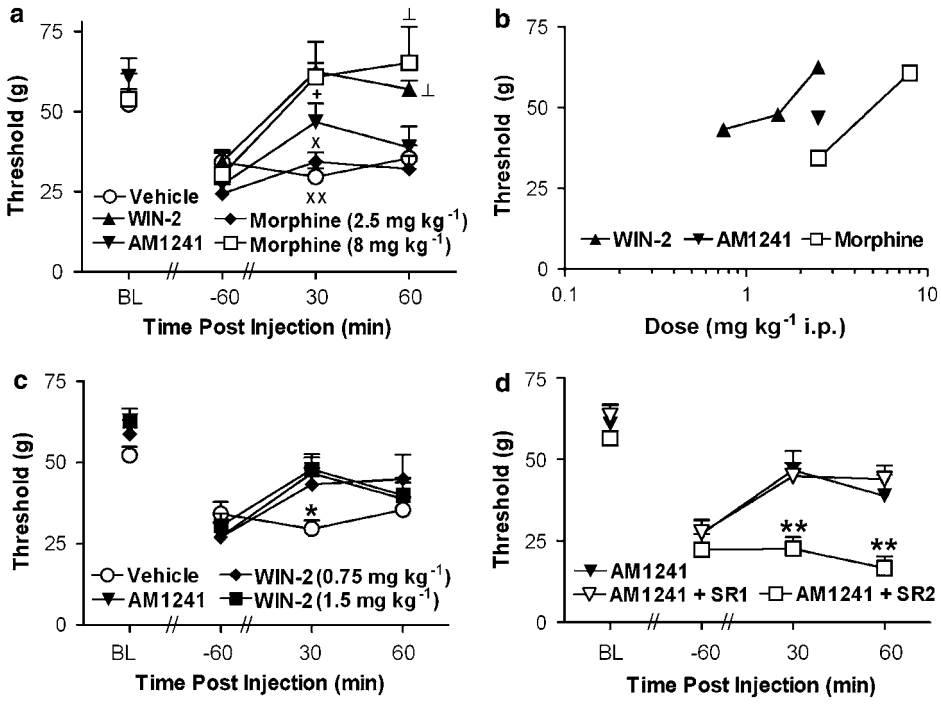

Figure 4 (**a**) WIN55,212-2 (WIN-2; 2.5 mg kg^{-1} i.p.) and morphine (8 mg kg^{-1} i.p.) reverse vincristine-evoked mechanical allodynia. The anti-allodynic effects of WIN55,212-2 (2.5 mg kg^{-1} i.p.) were similar to that of a high dose of morphine (8 mg kg^{-1} i.p.) and outlasted those of AM1241 (2.5 mg kg^{-1} i.p.) or a low dose of morphine (2.5 mg kg^{-1} i.p.). (**b**) Log-transformed dose–response curves for data shown in panel **a**. (**c**) AM1241 and WIN55,212-2 (1.5 and 0.75 mg kg^{-1} i.p.) produce similar suppressions of vincristine-induced mechanical hypersensitivity. (**d**) The AM1241-induced suppression of vincristine-induced hypersensitivity was blocked by the CB_2 antagonist SR144528 (SR2; 2.5 mg kg^{-1} i.p.) but not by the CB_1 antagonist SR141716 (SR1; 2.5 mg kg^{-1} i.p.). Data are means ± s.e.m. $^{**}P<0.01$, $^{*}P<0.05$ different from all groups, $^{xx}P<0.01$, $^{x}P<0.05$ different from AM1241, morphine (8 mg kg^{-1} i.p.) and WIN55,212-2 (2.5 mg kg^{-1} i.p.), $^{+}P<0.05$ different from WIN55,212-2 (2.5 mg kg^{-1} i.p.), $^{\perp}P<0.05$ different from AM1241, morphine (2.5 mg kg^{-1} i.p.) and vehicle (ANOVA and Fisher's PLSD *post hoc* test). $N=4$–8 per group.

24 h following the last injection of vincristine ($F_{1,16}=26.36$, $P<0.0002$, Figure 2b).

Assessment of spinal site of cannabinoid action

Mechanical withdrawal thresholds did not differ between vincristine-treated groups receiving the β-cyclodextrin vehicle (i.t.) and controls that were surgically implanted with catheters but did not receive an injection (i.t.). Therefore, these groups were pooled into a single control group for subsequent statistical analysis of drug effects. In vincristine-treated rats, administration of the CB_1/CB_2 agonist WIN55,212-2 (10 and 30 μg i.t.) increased mechanical withdrawal thresholds relative to either the control condition ($F_{2,19}=11.499$, $P<0.0006$, Figure 5b) or to day 12 preinjection levels ($F_{6,57}=2.698$, $P<0.04$; Figure 5b). *Post hoc* analyses failed to discriminate between the two doses of WIN55,212-2 (10 and 30 μg i.t.) at any time point.

The WIN55,212-2-induced increase in mechanical withdrawal thresholds was receptor-mediated ($F_{2,19}=7.152$, $P<0.005$; Figure 5c). WIN55,212-2 (10 μg i.t.) suppressed vincristine-evoked mechanical hypersensitivity relative to treatment with its receptor-inactive enantiomer WIN55,212-3 (10 μg, i.t) or the control condition ($P<0.02$ for each comparison). Mechanical withdrawal thresholds in WIN55,212-3-treated animals did not differ from control levels at any time point (Figure 5c).

Spinal administration of either SR141716 (30 μg i.t.) or SR144528 (30 μg i.t.) did not alter paw withdrawal thresholds relative to the control condition (Figure 6a). However, coadministration (i.t.) of both SR141716 and SR144528 concurrently with WIN55,212-2 blocked the cannabinoid-induced suppression of vincristine-evoked mechanical allodynia ($F_{4,33}=4.503$, $P<0.006$, $P<0.05$ for each comparison; Figure 6b). By contrast, a trend toward partial blockade of WIN55,212-2-induced anti-allodynia was observed following i.t. administration of the agonist with either the CB_1 ($P<0.13$) or CB_2 ($P<0.08$) antagonist alone, respectively. Planned comparisons confirmed that the CB_2 antagonist induced a partial blockade of the anti-allodynic effects of WIN55,212-2 at 5 and 30 min post-injection ($P<0.05$ for each comparison). Intrathecal coadministration of both antagonists with WIN55,212-2 blocked the cannabinoid-induced suppression of vincristine-evoked mechanical hypersensitivity at all time points ($P<0.006$ for each comparison; Figure 6b).

Assessment of peripheral site of cannabinoid action

The i.pl. injection lowered mechanical withdrawal thresholds relative to day 12 preinjection levels ($F_{1,22}=7.47$; $P<0.02$; Figure 7), consistent with the development of hypersensitivity at the site of injection. Enhanced hypersensitivity was differentially observed in the injected paw

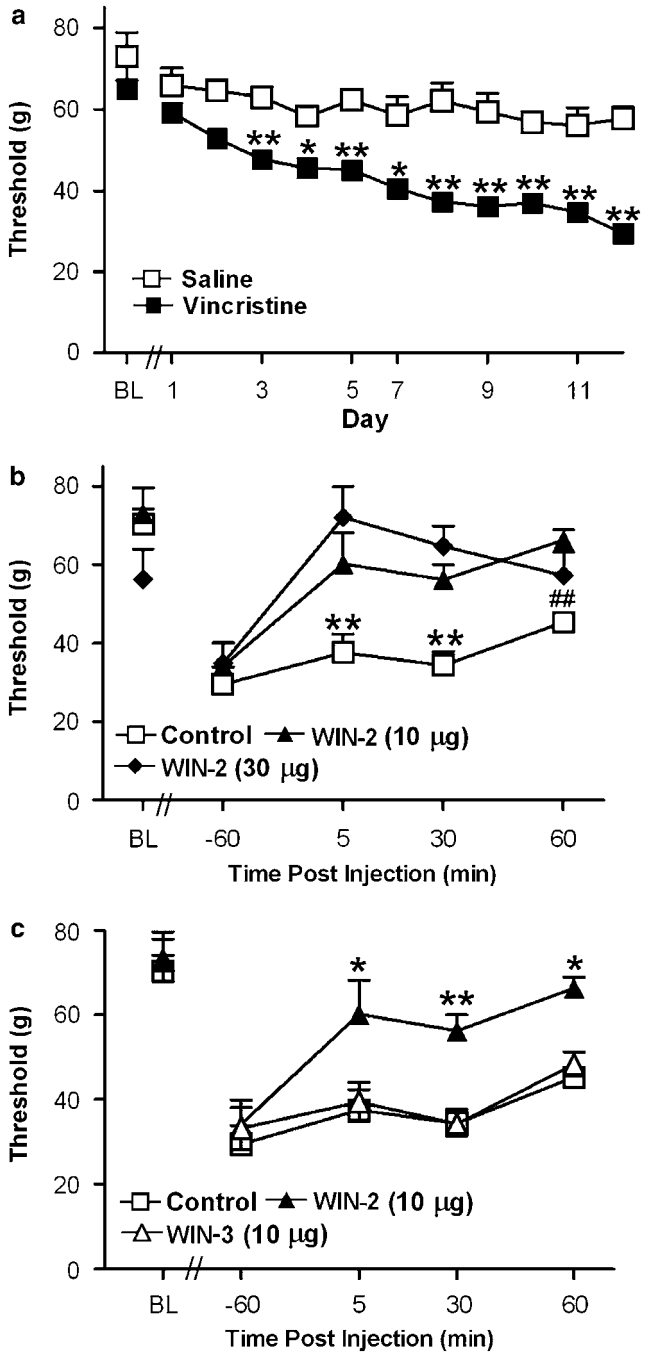

Figure 5 (**a**) Time course of development of vincristine-induced mechanical allodynia in rats implanted with i.t. catheters. (**b**) The CB_1/CB_2 agonist WIN55,212-2 (WIN-2; 10 and 30 μg i.t.) suppressed vincristine-induced mechanical allodynia. (**c**) WIN55,212-2 (10 μg i.t.) suppressed vincristine-evoked mechanical allodynia relative to the receptor-inactive enantiomer WIN55,212-3 (WIN-3; 10 μg i.t.) or the control condition. Data are means$\pm$s.e.m. $^{**}P<0.01$, $^{*}P<0.05$ different from all groups, $^{\#\#}P<0.01$ different from WIN55,212-2 (10 μg i.t.) (ANOVA and Fisher's PLSD *post hoc* test). $N=6$–9 per group.

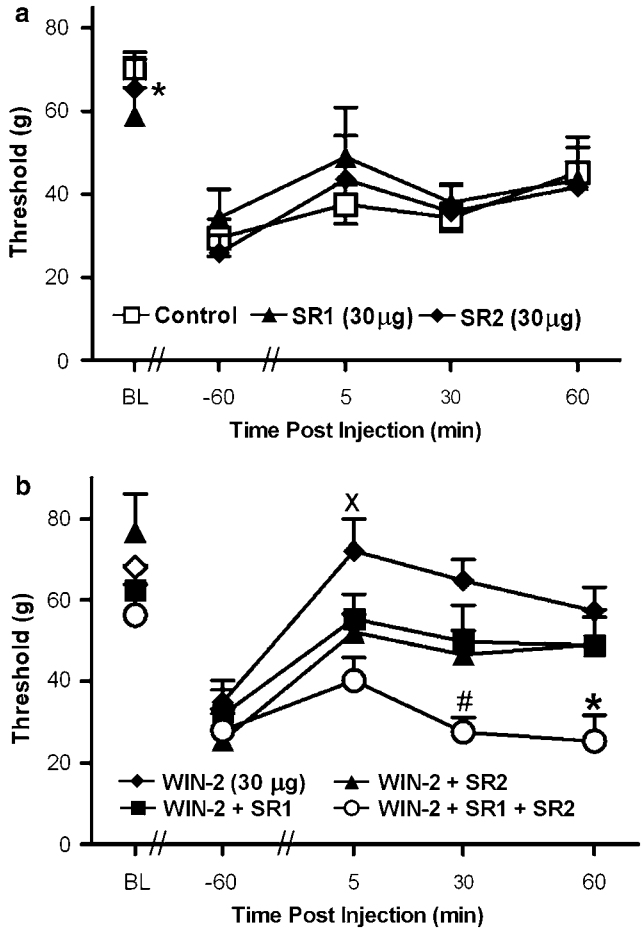

Figure 6 (**a**) The CB_1 antagonist SR141716 (SR1; 30 μg i.t.) and the CB_2 antagonist SR144528 (SR2; 30 μg i.t.) did not alter vincristine-induced mechanical allodynia relative to vehicle. (**b**) WIN55,212-2 (WIN-2; 30 μg i.t.) increased mechanical withdrawal thresholds relative to all other groups. Concurrent (i.t.) administration of SR141716 and SR144528 blocked the WIN55,212-2-induced suppression of vincristine-evoked mechanical allodynia. Data are mean $\pm$s.e.m. $^{*}P<0.05$ different from all groups, $^{\#}P<0.05$ different from WIN55,212-2 + SR2 and WIN55,212-2 (30 μg i.t.) $^{X}P<0.05$ different from WIN55,212-2 + SR2 and WIN55,212-2 + SR1 + SR2 (ANOVA and Fisher's PLSD *post hoc* test). $N=58$ per group.

($F_{2,22}=7.699$; $P<0.003$) in groups receiving vehicle ($P<0.02$) or the lower dose of WIN55,212-2 ($P<0.0003$) but not in groups receiving the high dose of WIN55,212-2. Paw withdrawal thresholds were also elevated relative to preinjection levels ($F_{1,22}=43.253$, $P<0.0002$) and this elevation differed as a function of the experimental treatment ($F_{2,22}=10.607$, $P<0.0007$; Figure 7). Unilateral injections of WIN55,212-2 (30 and 150 μg i.pl.) increased paw withdrawal thresholds in the non-injected paw relative to preinjection thresholds assessed immediately before the i.pl. injection ($P<0.01$ for each comparison).

Paw withdrawal thresholds were higher in the non-injected relative to the injected paw in all groups ($F_{1,22}=74.589$, $P<0.0002$; Figure 7). Paw withdrawal thresholds in the non-injected paw were similarly elevated ($F_{2,22}=8.76$, $P<0.002$) in groups receiving either dose of WIN55,212-2 (30 or 150 μg i.pl.) relative to groups receiving vehicle ($P<0.002$ for each comparison). Withdrawal thresholds in the non-injected paw were also altered relative to baseline levels ($P<0.0001$), and the magnitude of this change differed with the experimental treatment ($F_{2,22}=7.356$, $P<0.004$; Figure 7). Paw withdrawal thresholds in the non-injected paw were

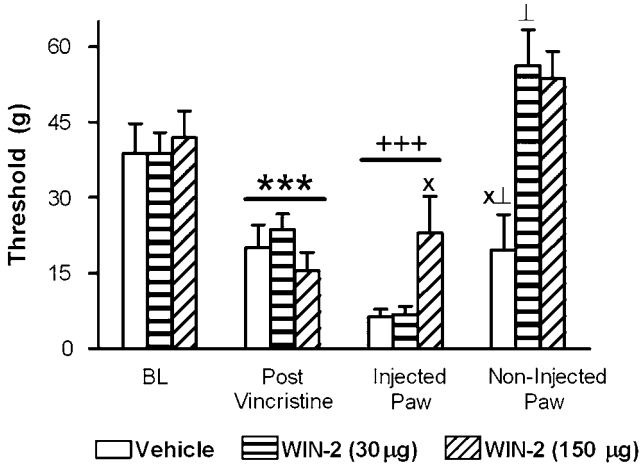

Figure 7 Local administration of the CB_1/CB_2 agonist WIN55,212-2 (WIN-2; 30 μg or 150 μg i.pl.) failed to suppress vincristine-induced mechanical hypersensitivity in the injected paw. Hypersensitivity was observed at the site of local injection following vehicle or WIN55,212-2 (30 μg i.pl.) administration relative to post-vincristine thresholds. Paw withdrawal thresholds in the non-injected paw were elevated relative to the injected paw in all groups. Data are means$\pm$s.e.m. ***$P<0.05$ different from baseline, post-i.pl.-injection and non-injected paw thresholds $^{+++}P<0.05$ different from baseline and non-injected paw thresholds, $^{x}P<0.05$ different from all groups for the same comparison (ANOVA, and Fisher's PLSD *post hoc* test), $^{\perp}P<0.05$ different from corresponding group baseline previncristine threshold measures (*t*-test). $N=7$–9 per group.

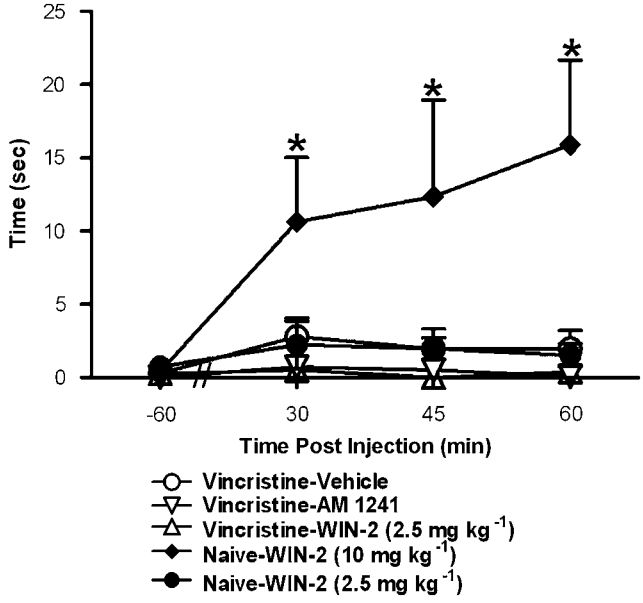

Figure 8 Anti-allodynic doses of AM1241 (2.5 mg kg^{-1} i.p.) and WIN55,212-2 (2.5 mg kg^{-1} i.p.) failed to induce catalepsy in vincristine-treated rats. In otherwise naive rats, WIN55,212-2 induced (10 mg kg^{-1} i.p.) catalepsy, as defined as an increase in time spent immobile in the bar test, at all post-injection time points. Data are means$\pm$s.e.m. *$P<0.05$ different from all groups, (ANOVA and Fisher's PLSD *post hoc* test). $N=6$ per group.

higher than baseline in groups receiving WIN55,212-2 (30 μg i.pl.; $P<0.03$) and lower than baseline levels in groups receiving the vehicle (i.pl.). A trend ($P<0.08$, *t*-test) towards elevated paw withdrawal thresholds in the non-injected paw relative to baseline was also observed in groups receiving WIN55,212-2 (150 μg i.pl.). By contrast, paw withdrawal thresholds in the injected paw were lower than baseline ($P<0.0002$) for all groups.

Local injection of WIN55,212-2 (30 μg i.pl.) did not alter mechanical withdrawal thresholds in the injected paw relative to vehicle. By contrast, WIN55,212-2 (150 μg i.pl.) elevated mechanical withdrawal thresholds in the injected paw relative to either the vehicle or lower dose of WIN55,212-2 (30 μg i.pl) ($F_{2,22}=4.083$, $P<0.05$; $P<0.03$ for all comparisons; Figure 7) without suppressing vincristine-induced mechanical hypersensitivity. WIN55,212-2 also failed to suppress vincristine-evoked mechanical allodynia at the site of i.pl. injections relative to day 12 thresholds (observed before i.pl. injection) at any dose.

Assessment of catalepsy

Systemic doses of WIN55,212-2 (2.5 mg kg^{-1} i.p.) and AM1241 (2.5 mg kg^{-1} i.p.) that suppressed vincristine-evoked mechanical allodynia were compared with a dose of WIN55, 212-2 (10 mg kg^{-1} i.p.) known to impair motor activity (Figure 8). WIN55,212-2-induced (10 mg kg^{-1} i.p.) catalepsy in the bar test ($F_{4,25}=4.34$, $P<0.01$; Figure 8) relative to all other conditions ($P<0.05$ for all comparisons) or preinjection levels ($F_{12,75}=3.783$, $P<0.004$). Neither WIN55,212-2 nor AM1241, administered at doses that suppressed vincristine-evoked mechanical allodynia, suppressed motor activity in the bar test (Figure 8).

Discussion

Vincristine preferentially induces behavioural sensitization to mechanical as opposed to thermal stimulation

Activation of cannabinoid CB_1 and CB_2 receptor subtypes attenuates vincristine-induced mechanical hypersensitivity. Using the vincristine injection paradigm employed here, animals remained in relatively good health, as characterized by the absence of mortality observed with higher dosing paradigms (Authier *et al*., 1999, 2003a). Vincristine induced a failure of normal weight gain relative to saline-treated controls, similar to previous reports (Weng *et al*., 2003). A small percentage of animals (<5%) exhibited gastrointestinal bleeding, a common problem for chemotherapy patients (Sandler *et al*., 1969; Jackson *et al*., 1988; Tolstoi, 2002; Ozcay *et al*., 2003), during later stages of the experiment (that is, days 5–12). Weng *et al*. (2003) reported no similar symptoms and normal stool in the same vincristine-dosing paradigm. Differences may be attributed to the large number of subjects evaluated in our study coupled with the low frequency of symptom occurrence.

Changes in mechanical withdrawal thresholds observed here cannot be attributed to the development of sensitization to repeated testing. Mechanical allodynia developed in vincristine-treated animals, but not in their saline-treated counterparts who were tested at the same time. Mechanical hypersensitivity developed by day 3 post-vincristine, reaching

its lowest level on day 7 and remained stable until day 12. Other studies similarly report that mechanical hypersensitivity is maximal by day 8 post-vincristine (Nozaki-Taguchi *et al.*, 2001; Weng *et al.*, 2003). Vincristine-induced mechanical allodynia resolved completely by day 31 in our study, although lack of recovery has been reported with other dosing paradigms (Nozaki-Taguchi *et al.*, 2001).

Hypersensitivity to thermal stimulation (or thermal hyperalgesia) was notably absent in vincristine-treated rats that nonetheless exhibited robust mechanical allodynia. By contrast, paclitaxel induces thermal hyperalgesia or thermal hypoalgesia (depending upon the dosing schedule), which may be absent in vincristine and cisplatin models of chemotherapy-induced neuropathy (Authier *et al.*, 2000, 2003a, b; Nozaki-Taguchi *et al.*, 2001; Weng *et al.*, 2003; Lynch *et al.*, 2004; Cata *et al.*, 2006a). Thermal hyperalgesia has been observed in mice using a different vincristine dosing paradigm beginning at 4 weeks following initial vincristine treatment (Kamei *et al.*, 2005). Nonetheless, vincristine may induce cold allodynia/hyperalgesia (Authier *et al.*, 2003b; Lynch *et al.*, 2004), consistent with clinical reports (Cata *et al.*, 2006b).

An upregulation of neuropeptide Y (NPY) in medium and large diameter dorsal root ganglion cells has been postulated to underlie development of mechanical allodynia (in the absence of thermal hyperalgesia) following spinal nerve ligation (Ossipov *et al.*, 2002). More work is necessary to determine whether similar neurochemical changes accompany the development of vincristine-evoked mechanical allodynia in our study.

Subtype specificity of cannabinoid anti-allodynic actions

WIN55,212-2 ($2.5\,mg\,kg^{-1}$ i.p.) restored mechanical withdrawal thresholds to >100% of previncristine levels. WIN55,212-2 ($1.5\,mg\,kg^{-1}$ i.p.) reversed both mechanical and thermal hypersensitivity in a paclitaxel-induced neuropathy model (Pascual *et al.*, 2005) but did not reverse vincristine-induced mechanical hypersensitivity in our study. Doses of WIN55,212-2 that eliminated vincristine-induced mechanical allodynia in our study did not induce motor deficits in the bar test. Thus, WIN55,212-2-induced anti-allodynic effects are independent of any motor effects of cannabinoids. Similar or higher doses of WIN55,212-2 ($2.5-5\,mg\,kg^{-1}$ i.p.) also attenuate mechanical allodynia in models of traumatic nerve injury (Herzberg *et al.*, 1997; Bridges *et al.*, 2001; Fox *et al.*, 2001; Ibrahim *et al.*, 2003; Walczak *et al.*, 2005; LaBuda and Little, 2005) and diabetic neuropathy (Ulugol *et al.*, 2004). WIN55,212-2 also attenuates deep tissue hyperalgesia in a murine model of cancer pain through a CB_1 mechanism (Kehl *et al.*, 2003).

AM1241 ($2.5\,mg\,kg^{-1}$ i.p.) induced a CB_2-mediated suppression of vincristine-induced mechanical allodynia without inducing antinociception. Metabolism of AM1241 may limit the duration of CB_2-mediated anti-allodynia observed here. Nonetheless, CB_2 agonists may represent preferred therapeutic agents relative to CB_1 agonists due to their limited profile of CNS side-effects (Hanus *et al.*, 1999; Malan *et al.*, 2001). AM1241 is an effective anti-hyperalgesic agent in animal models of traumatic nerve injury (Ibrahim *et al.*, 2003) and inflammation (Quartilho *et al.*, 2003; Hohmann *et al.*, 2004; Nackley *et al.*, 2003, 2004). Our studies suggest that CB_2 is also a novel target for the treatment of chemotherapy-induced neuropathy.

Activation of either CB_1 or CB_2 receptors suppressed the maintenance of vincristine-evoked mechanical allodynia. The anti-allodynic effects of WIN55,212-2 were partially blocked by each antagonist alone at 30 min post-injection whereas complete blockade was observed at 60 min post-drug. Moreover, i.t. administration of both antagonists concurrently completely blocked the anti-allodynic effects of spinally administered WIN55,212-2. Our data also raise the possibility that targeting multiple cannabinoid receptor subtypes simultaneously may act synergistically to suppress chemotherapy-induced neuropathy.

Effects of cannabinoids and morphine on vincristine-induced neuropathy

Opiates are commonly administered to cancer patients experiencing chemotherapy-induced neuropathy (Lynch *et al.*, 2004; Cata *et al.*, 2006b). In our study, a leftward shift in the dose–response curve for mechanical withdrawal thresholds was observed for WIN55,212-2 relative to morphine. WIN55,212-2, at a dose of $2.5\,mg\,kg^{-1}$, exhibited effects of approximately the same magnitude as morphine at a dose of $8\,mg\,kg^{-1}$. Additional doses are required to enable calculations of the ED_{50} for each drug and verify differences in agonist potency. Our low dose of morphine ($2.5\,mg\,kg^{-1}$ i.p.) suppressed neuropathic nociception induced by spinal nerve ligation (LaBuda and Little, 2005; Joshi *et al.*, 2006) and induced antinociception (Ibrahim *et al.*, 2006), but failed to suppress vincristine-induced allodynia in our study. The high dose of morphine ($8\,mg\,kg^{-1}$ i.p.) normalized paw withdrawal thresholds in our study but only partially (50%) reversed paclitaxel-evoked mechanical hypersensitivity (Flatters and Bennett, 2004). Cannabinoids show enhanced antihyperalgesic efficacy relative to opiates in other neuropathic pain models (Mao *et al.*, 1995, 2000). Lower efficacy of morphine in reducing abnormal sensations related to myelinated as opposed to unmyelinated fibre activation (Taddese *et al.*, 1995) is consistent with the differential neuroanatomical distribution of μ-opioid and cannabinoid receptors at spinal and primary afferent levels (Hohmann and Herkenham, 1998a; Hohmann *et al.*, 1999; Bridges *et al.*, 2001). Thus, cannabinoids may be more potent and efficacious than opiates in suppressing diverse forms of neuropathic and deafferentation-induced pain.

Mechanisms and site of action

In our study, WIN55,212-2 suppressed vincristine-induced mechanical allodynia when administered i.t. but not when administered locally into the paw. In fact, local injections of either vehicle or WIN55,212-2 ($30\,\mu g$ i.pl.) in our study *enhanced* mechanical allodynia in the injected paw relative to preinjection levels. Changes in weight bearing due to sensitization at the site of i.pl. injection may contribute to the increases in paw withdrawal thresholds observed in all groups (including vehicle) in the non-injected paw. The

same local dose employed here (30 μg i.pl.) suppressed mechanical allodynia in models of diabetic neuropathy (Ulugol *et al.*, 2004) and traumatic nerve injury (Fox *et al.*, 2001) but failed to attenuate paclitaxel neuropathy (Pascual *et al.*, 2005) or suppress vincristine-induced neuropathy in our study. Local injection of WIN55,212-2 (30 μg i.pl.) also elevated paw withdrawal thresholds in the non-injected paw above baseline (previncristine) levels, but failed to reverse the hypersensitivity observed at the site of the i.pl. injection. Leakage of the cannabinoid into the systemic circulation may contribute to changes in paw withdrawal thresholds observed in the non-injected paw. A higher local WIN55,212-2 dose (150 μg i.pl.) that induces clear systemic effects (Fox *et al.*, 2001) eliminated the hypersensitivity observed at the site of the i.pl. injection. However, this dose nonetheless failed to suppress vincristine-evoked mechanical allodynia relative to preinjection levels and did not normalize paw withdrawal thresholds to previncristine levels.

Our data provide direct evidence that spinal sites of action are implicated in both CB_1 and CB_2 receptor-mediated suppressions of chemotherapy-induced neuropathy. Interestingly, CB_2 receptor mRNA and protein are upregulated in spinal cord of rats subjected to traumatic nerve injury (Zhang *et al.*, 2003; Walczak *et al.*, 2005; Wotherspoon *et al.*, 2005). Direct spinal administration of a CB_2 agonist also suppresses mechanically evoked responses in wide dynamic range neurons in neuropathic but not in sham-operated rats (Sagar *et al.*, 2005), suggesting a functional role for spinal CB_2 receptors in neuropathic pain states.

Vincristine induces central sensitization in spinal wide dynamic range neurons, including abnormal spontaneous activity, wind-up and afterdischarge responses to suprathreshold mechanical stimulation (Weng *et al.*, 2003). These aberrant neurophysiological responses may mediate the observed chemotherapy-induced neuropathy. Cannabinoids suppress C-fibre-mediated responses and wind-up of spinal wide dynamic range neurons through either CB_1 (Strangman and Walker, 1999; Drew *et al.*, 2000) or CB_2 (Nackley *et al.*, 2004)-specific mechanisms. Further studies are required to determine the neurophysiological basis for cannabinoid-mediated suppression of chemotherapy-induced neuropathy (see Hohmann, 2005).

Enhanced primary afferent glutamate release (presynaptic facilitation) may also contribute to the abnormal behavioural phenotype and central sensitization induced by chemotherapeutic treatment. Consistent with this hypothesis, decreased protein levels for the glutamate-aspartate transporter (GLAST), glial glutamate transporter-1 (GLT-1) and excitatory amino-acid carrier-1 (EAAC1) are observed following paclitaxel treatment (Cata *et al.*, 2006a). It is worth noting, however, that glutamate and NMDA receptor antagonists reverse hyperalgesia in a nerve-injury model (Mao *et al.*, 1995), but not in chemotherapy-induced neuropathy models (Aley and Levine, 2002; Flatters and Bennett, 2004). Thus, distinct mechanisms may be implicated in the development of neuropathic nociception induced by traumatic nerve injury and chemotherapeutic treatment, respectively.

Abnormal primary afferent input, presynaptic and/or descending (Porreca *et al.*, 2001; Vera-Portocarrero *et al.*, 2006) facilitation and chemotherapy-induced dysregulation of calcium homoeostasis (Siau and Bennett, 2006) may enhance neuronal excitability, thereby increasing intracellular Ca^{2+} (Kawamata and Omote, 1996). Ethosuximide, a T-type calcium antagonist and other drugs which reduce intra- and extracellular Ca^{2+}, also reduce vincristine-induced mechanical hypersensitivity (Flatters and Bennett, 2004; Siau and Bennett, 2006). Additional studies are required to determine if cannabinoid suppression of chemotherapy-induced neuropathy is related to cannabinoid suppression of Ca^{2+} conductance (Mackie and Hille, 1992; Mackie *et al.*, 1995) and central sensitization.

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Conflict of interest

AM serves as a consultant for MAK Scientific.

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