British Journal of Pharmacology (2001) 133, 586–594

www.nature.com/bjp

The synthetic cannabinoid WIN55,212-2 attenuates hyperalgesia and allodynia in a rat model of neuropathic pain

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1 The analgesic properties of the synthetic cannabinoid WIN55,212-2 were investigated in a model of neuropathic pain. In male Wistar rats, bilateral hind limb withdrawal thresholds to cold, mechanical and noxious thermal stimuli were measured. Following this, unilateral L5 spinal nerve ligation was performed. Seven days later, sensory thresholds were reassessed and the development of allodynia to cold and mechanical stimuli and hyperalgesia to a noxious thermal stimulus confirmed.

2 The effect of WIN55,212-2 (0.1–5.0 mg kg^{-1}, i.p.) on the signs of neuropathy was then determined; there was a dose related reversal of all three signs of painful neuropathy at doses which did not generally alter sensory thresholds in the contralateral unligated limb. This effect was prevented by co-administration of the CB_1 receptor antagonist SR141716a, but not by co-administration of the CB_2 receptor antagonist SR144528, suggesting this action of WIN55,212-2 is mediated *via* the CB_1 receptor. Administration of SR141716a alone had no affect on the observed allodynia and hyperalgesia, which does not support the concept of an endogenous analgesic tone.

3 These data indicate that cannabinoids may have therapeutic potential in neuropathic pain, and that this effect is mediated through the CB_1 receptor.

British Journal of Pharmacology (2001) **133,** 586–594

Keywords: Cannabinoid; analgesia; neuropathic pain; hyperalgesia; allodynia; WIN55,212-2; CB_1 receptor; SR141716a

Abbreviations: 2-AG, 2-arachidonyl glycerol; CB_1, cannabinoid 1 receptor; CB_2, cannabinoid 2 receptor; CCI, chronic constriction injury; DMSO, dimethylsulfoxide; PEA, palmitoylethanolamide; PNL, partial nerve ligation; SNL, spinal nerve ligation; SR1, SR141716a; SR2, SR144528; THC, Δ^9-tetrahydrocannabinol

Introduction

There have been suggestions that extracts of *Cannabis sativa* have medicinal properties for thousands of years, but until recently, little sound scientific data has been available to support this hypothesis. However, the structure of Δ^9-tetrahydrocannabinol (THC), the major psychoactive component of the 66 known cannabinoids found in marijuana, was elucidated in the early 1960s and since then THC has been shown to be associated with a number of pharmacological effects, including analgesia (Gaoni & Mechoulam, 1964). A further breakthrough in the elucidation of cannabinoid pharmacology came in 1988 with the discovery of a cannabinoid receptor in rat brain (CB_1, Devane *et al*., 1988). This receptor was subsequently cloned in 1990 (Matsuda *et al*., 1990) and was shown to have a seven transmembrane G-protein coupled structure (Howlett *et al*., 1986). A second cannabinoid receptor (CB_2), with 44% sequence homology to CB_1, was identified and cloned in 1993 (Munro *et al*., 1993) and was found to be predominantly expressed by cells of the immune system. Also in 1992, the endogenous cannabinoid anandamide (AEA) was extracted from the brain and spinal cord (Devane *et al*., 1992). More recently palmitoylethanolamide (PEA) and 2-arachidonyl glycerol (2-AG) have also been identified as potential endogenous cannabinoids (Mechoulam *et al*., 1995; Sugiura *et al*., 1995). The synthesis of the specific high affinity receptor antagonists, SR141716a (SR1) at CB_1 (Rinaldi-Carmona *et al*., 1995; Welch *et al*., 1998) and SR144528 (SR2) at CB_2 (Rinaldi-Carmona *et al*., 1998; Griffin *et al*., 1999), and the development of CB_1 (Ledent *et al*., 1999) and CB_2 'knockout mice' (Buckley *et al*., 2000) afforded new techniques for the elucidation of CB receptor mediated effects. Several studies have demonstrated an analgesic effect with synthetic and endogenous cannabinoids in inflammatory pain models (for example Jaggar *et al*., 1998a, b; Calignano *et al*., 1998; Richardson *et al*., 1998.)

Neuropathic pain is defined as 'pain initiated or caused by a primary lesion or dysfunction in the nervous system' (Merskey & Bogduk, 1994) and is an area of largely unmet therapeutic need. Tricyclic antidepressants and certain anticonvulsants are the mainstay of clinical therapy for neuropathic pain (McQuay *et al*., 1996; Sindrup & Jensen, 1999). However, systematic reviews reveal that only between 30–50% of patients suffering from neuropathic pain achieve clinically significant (>50%) pain relief with any available single therapy (McQuay *et al*., 1996; Sindrup & Jensen, 1999). Furthermore, side effects of these therapies often limit their usefulness (McQuay *et al*., 1996). Although controversial, it is generally accepted that opioids are less effective in neuropathic than inflammatory pain. (Rowbotham, 1999). One explanation for this observation is that spinal opioid

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receptor expression decreases after peripheral nerve injury (Besse *et al*., 1992). A recent study has demonstrated little decrease of spinal CB receptor binding when compared to μ-opioid receptor binding after neonatal capsaicin treatment (Hohmann & Herkenham, 1998). Furthermore, it has also been demonstrated using immunocytochemistry that, following dorsal rhizotomy, spinal CB_1 receptor expression remains unaltered (Farquhar-Smith *et al*., 2000). This sparing of CB_1 receptors following peripheral nerve injury could indicate a potential therapeutic advantage of cannabinoids over opioids in the treatment of neuropathic pain. This hypothesis is further supported by a recent study (Mao *et al*., 2000), which demonstrated an anti-hyperalgesic effect of THC in an animal model of neuropathic pain and suggested a therapeutic advantage of THC over opioids in painful neuropathy.

Various animal models of neuropathic pain have been developed (Kim *et al*., 1997). The three most commonly used models share partial injury of the sciatic nerve and a subsequent alteration in hind limb withdrawal thresholds to sensory stimuli as a common feature. In particular, hyperalgesia to a noxious thermal stimulus and allodynia to cold and mechanical stimuli are usually observed. The first model described was the chronic constriction injury (CCI) model in which loose chromic gut ligatures are placed around the sciatic nerve (Bennett & Xie, 1988). A more recent modification was the partial sciatic nerve ligation (PNL) model, in which a tight ligation is placed around 1/3 to 1/2 of the sciatic nerve trunk (Seltzer *et al*., 1990). A third model is the spinal nerve ligation (SNL) model, in which the L5 and L6 spinal nerves are tightly ligated (Kim & Chung, 1992). A direct comparison of various features of these three models of neuropathic pain has been reported (Kim *et al*., 1997). In this study, the latency and duration of signs of painful neuropathy were examined, and the authors also examined the effect of sympathectomy on these signs. This study showed that the partial nerve injury evoked signs with roughly the same onset in all three models, but that qualitatively the mechanical and cold allodynia were greatest in SNL. Ongoing pain, as determined by non-weight bearing behaviour, was greatest in CCI. Surgical lumbar sympathectomy caused a reduction in pain behaviour in all three models, with the largest decrease in SNL, suggesting a greater sympathetic nervous system involvement in this model.

There are two reports of the effectiveness of cannabinoids in an animal model of neuropathic pain. One study reported that the synthetic cannabinoid WIN55,212-2 alleviated the allodynia and hyperalgesia associated with the CCI model of neuropathic pain (Herzberg *et al*., 1997). It was confirmed that this effect was mediated through the CB_1 receptor and occurred at a dose that was not associated with obvious side effects. This study also demonstrated an increase in thermal hyperalgesia and mechanical allodynia by administration of SR141716a alone, leading the authors to infer the presence of an endogenous tone of cannabinoid analgesia in neuropathy. However, the evidence for such tone during inflammation is controversial (Beaulieu *et al*., 2000). A separate study reported that THC, administered intrathecally, also alleviated the hyperalgesia of the CCI model (Mao *et al*., 2000). This effect was shown to be CB_1 receptor mediated.

It has been suggested that the neuropathy in the CCI model is largely dependent upon an inflammatory reaction (Wagner *et al*., 1998) and therefore the anti-inflammatory effects of cannabinoids may have obfuscated the true effect on neuropathy in this model. Both the PNL and SNL are associated with a lesser inflammatory component. Therefore, in this study we examined whether the anti-allodynic and anti-hyperalgesic effects of cannabinoids were found in a model of neuropathic pain associated with less of an inflammatory component than the CCI model, namely the SNL model. We also investigated the concept of endogenous tone in this model.

Methods

Animal maintenance

All experiments were approved by the Home Office. Animals were housed, six per cage at constant temperature under a 14 : 10 h light/dark cycle, with free access to food and water at all times.

Surgery

A left L5 spinal nerve ligation, a modification of that described by Kim & Chung (1992), was performed on male Wistar rats of between 200–350 g in weight ($n=126$). The rats were anaesthetized (pentobarbitone sodium, 60 mg kg^{-1}, i.p.) and the surgery performed using standard aseptic techniques. Using the transverse processes of L6 as a guide, the left paraspinal muscles were exposed and separated from the spinous processes of L4 to S2 by blunt dissection. The L6 transverse process was then removed by hemi-laminectomy and the L5 spinal nerve exposed and identified according to its size and position. This was then ligated tightly with a 3-0 silk suture and sectioned 1–2 mm distal to the suture before haemostasis was confirmed and the wound was sutured at both muscle and skin levels. Sham surgery ($n=6$) was performed by exposing the L5 spinal nerve as described above, but not damaging it.

Sensory testing

Three tests of hind limb withdrawal to thermal, cold and mechanical stimuli were employed in this study. Each test was repeated on both the operated hind paw and the contralateral hind paw with all sensory testing performed by a 'blinded' investigator.

(i) Cold allodynia was assessed using the acetone drop application technique modified from Carlton *et al*. (1994). Animals were placed in plexiglass boxes ($23\times18\times14$ cm) with 0.8 cm diameter mesh flooring and allowed to acclimatize for 15 min or until exploratory behaviour ceased. Sampling was performed by the application of a single bubble of acetone to the mid plantar surface of each hind paw from the tip of a 1 ml syringe. A positive response was recorded if the animal withdrew the paw following application. For each measurement, the paw was sampled five times and a mean calculated. At least 3 min elapsed between each test.

(ii) Thermal hyperalgesia was assessed using an infrared noxious heat stimulus (Plantar test, Ugo Basile, Italy,

Hargreaves *et al*., 1988). Briefly, animals were placed in a clear plexiglass box ($23 \times 18 \times 14$ cm) with a dry glass floor and allowed to acclimatize for 15 min or until exploratory behaviour ceased. A focused beam of radiant heat at a constant temperature of 46°C and wavelength of 50 nm was applied to the plantar surface of the paw. The hind paw withdrawal latency (s) to this stimulus was tested three times at intervals of not less than 3 min and a mean calculated. The device has an automatic cut-off at 21 s to avoid the risk of thermal injury to the skin.

(iii) Mechanical allodynia was assessed using an electronic Von Frey device (Möller *et al*., 1998; Ahmad & Rice, 1999). Animals were placed into raised plexiglass boxes ($23 \times 18 \times 14$ cm) with 0.8 cm diameter mesh flooring and allowed to acclimatize for 15 min or until exploratory behaviour ceased. Sampling was conducted by a calibrated nylon electronic force transducer (1.0 mm diameter, type 739, Somedic Sales AB, Sweden) which was applied manually to the mid-plantar hind paw at a rate of 0.5–1.0 N s^{-1} with the withdrawal threshold amplified (Senselab 701, Somedic, Sweden) and displayed on a PC based chart recorder (AcqKnowledge v3.02, Biopac Systems Inc. U.S.A.). The mean withdrawal threshold was taken from a set of five applications, not less than 10 s apart.

Pharmacological interventions

Baseline sensory thresholds were measured for each group of animals ($n=6$) pre-operatively and 7 days post-operatively. Animals displaying thermal hyperalgesia or cold or mechanical allodynia were then administered the relevant drug according to a pre-determined randomization table and testing was re-performed at 20, 40, 60 and 90 min post drug administration. Each group of animals was used for only one drug administration protocol to ensure no 'carry over' effects, hence they were used for only a single treatment. WIN55,212-2 (0.1–5.0 mg kg^{-1}, i.p.) was administered at $t=0$, in 40% dimethylsulfoxide (DMSO) in saline solvent, SR141716a (0.5 mg kg^{-1}, i.p.) and SR144528 (1 mg kg^{-1}, i.p.) were administered at $t=-5$ min and dissolved in 40% DMSO in saline solvent. All animals were humanely culled at the end of the experiment. A summary of the treatment groups is displayed in Table 1.

Table 1 Summary of treatment groups for experimental procedures

$Group_{stimulus}$	*WIN55,212-2 dose* (mg kg^{-1}) *or Solvent* (40% DMSO)	*SR141716a* (0.5 mg kg^{-1}) *or SR144528* (1 mg kg^{-1})
1_{cold}, 2_{therm}	0.1 WIN	None
3_{cold}, 4_{therm}, 5_{mech}	0.5 WIN	None
6_{cold}, 7_{therm}, 8_{mech}	2.5 WIN	None
9_{mech}	5.0 WIN	None
10_{cold}, 11_{therm}	2.5 WIN	SR141716a
12_{mech}	5.0 WIN	SR141716a
13_{cold}	2.5 WIN	SR144528
14_{cold}, 15_{therm}, 16_{mech}	Solvent	None
17_{cold}, 18_{therm}, 19_{mech}	Solvent	SR141716a
20_{cold}, 21_{therm}, 22_{mech}	Sham surgery only, no drug testing	

Statistical analysis

Statistical significance was determined for neuropathy by a paired *t*-test and for drug effects by one-way ANOVA (Dunnett, compared to post operative values), both taking $P<0.05$ as statistically significant (SigmaStat v2.0, Jandel Corporation, U.S.A.)

Drugs

WIN55,212-2 was supplied by Tocris Cookson Ltd., U.K., SR141716a was a gift from SRI–NIMH chemical synthesis program and SR144528 was a gift from Sanofi Recherche, France.

Results

All animals included in the analysis of study exhibited altered sensory thresholds 7 days following SNL (rejection rate = 7%). In the sham surgery groups (20–22, Table 1) there was no significant difference from baseline sensory thresholds. In all animals in which solvent only was administered as a control, sensory thresholds were not subsequently altered in either the ipsilateral or contralateral paws.

WIN55,212-2 studies

(i) Administration of WIN55,212-2 reversed the cold allodynia produced by SNL at a dose of 2.5 mg kg^{-1} over 90 min (Figure 1). This effect was observed to be both dose- and time-dependent with a maximum effect at 20–40 min. The dose dependency was observed with a non-significant trend towards an effect at 0.5 mg kg^{-1} WIN, but no effect at 0.1 mg kg^{-1}.

(ii) WIN55,212-2 also reversed the thermal hyperalgesia produced by SNL. This effect was again observed to be both dose and time dependent with the results generally concurring with those from the cold allodynia studies (Figure 2). Thermal hyperalgesia was attenuated throughout the entire 90 min experiment at a dose of 2.5 mg kg^{-1} WIN55,212-2 and also between 20–40 min at a dose of 0.5 mg kg^{-1}. No effect was seen at the 0.1 mg kg^{-1} dose.

(iii) Mechanical allodynia produced by SNL was not as effectively attenuated by WIN55,212-2 as the cold allodynia and thermal hyperalgesia (Figure 3). A significant effect was only observed at the 5.0 mg kg^{-1} dose throughout the entire 90 min experiment. However, significant effects were also observed on the sensory thresholds of the contralateral limb at this dose. At 20 and 40 min post dosing the 2.5 and 0.5 mg kg^{-1} doses had no effect on mechanical allodynia.

Receptor involvement

The CB_1 receptor antagonist SR141716a (0.5 mg kg^{-1}) prevented the anti-hyperalgesic or anti-allodynic effects seen at the highest doses of WIN55,212-2 (Figure 4). In the mechanical and thermal stimuli studies, SR141716a prevented the anti-allodynic and anti-hyperalgesic effects of WIN55,212-

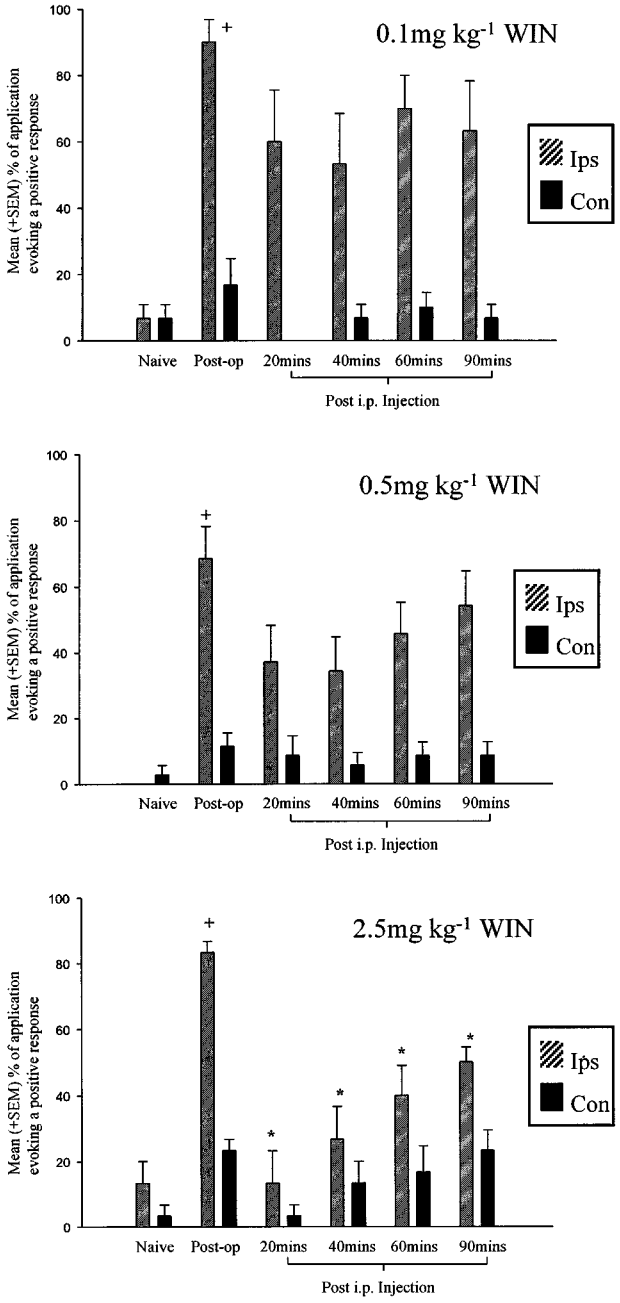

Figure 1 Bilateral hind limb withdrawal responses to cold stimulation (acetone drop) in rats rendered neuropathic by L5 spinal nerve ligation administered WIN55,212-2 (0.1–2.5 mg kg^{-1}, i.p., $n=6$ per dose). Attenuation of cold allodynia was observed at a dose of 2.5 mg kg^{-1} WIN55,212-2. $+P<0.05$, paired t-test, $*P<0.05$, one-way ANOVA (Dunnett's).

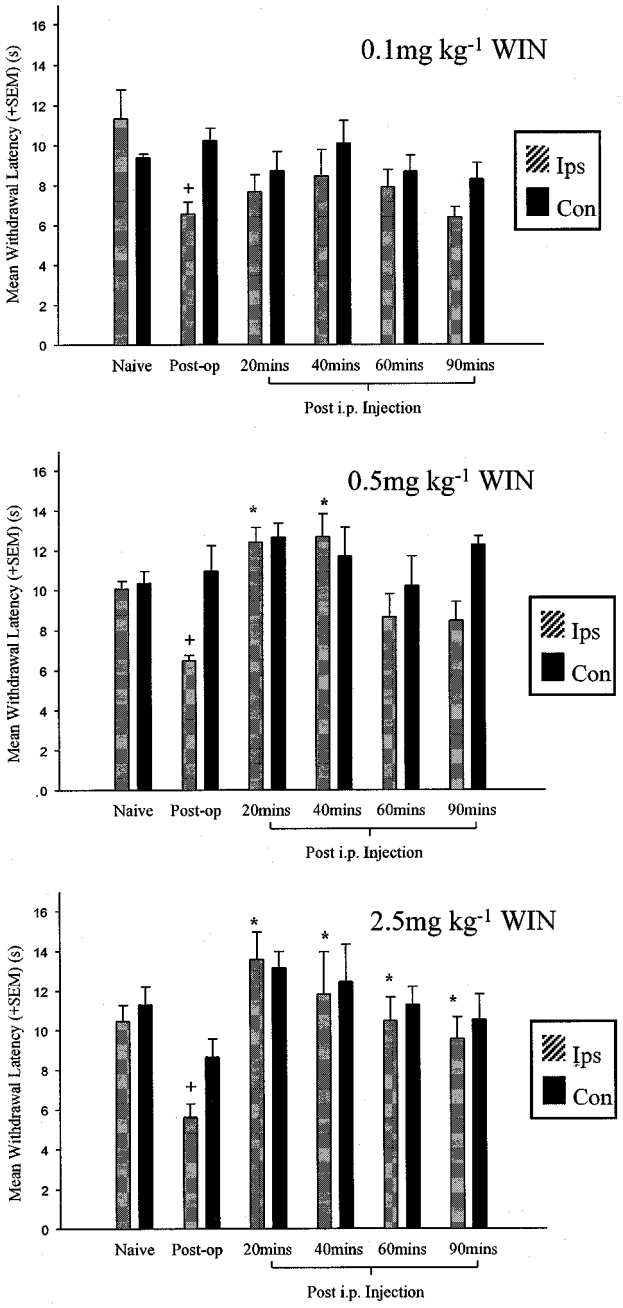

Figure 2 Bilateral hind limb withdrawal responses to thermal stimulation (Hargreaves' device) in rats rendered neuropathic by L5 spinal nerve ligation administered WIN55,212-2 (0.1–2.5 mg kg^{-1}, i.p., $n=6$ per dose). Attenuation of thermal hyperalgesia was observed at doses of 0.5 and 2.5 mg kg^{-1} WIN55,212-2. $+P<0.05$, paired t-test, $*P<0.05$, one-way ANOVA (Dunnett's).

2 over the 90 min investigation period. In the cold stimuli studies, the anti-allodynic effects of WIN55,212-2 were prevented over 40 min, but persisted at 60 and 90 min at a much reduced level than the 2.5 mg kg^{-1} alone group. Administration of SR141716a alone had no significant effects on any of the sensory thresholds (Figure 5). Co-administration of WIN55,212-2 (2.5 mg kg^{-1}) and the CB_2 receptor antagonist SR144528 (1 mg kg^{-1}) did not prevent the anti-allodynic effect of WIN55,212-2 in cold stimuli over 90 min (Figure 6).

Discussion

This study has provided evidence for analgesic actions of the synthetic cannabinoid WIN55,212-2 in the spinal nerve ligation model of painful neuropathy. WIN55,212-2 attenuated cold allodynia and thermal hyperalgesia at a dose of 2.5 mg kg^{-1}, i.p and mechanical allodynia at a dose of 5.0 mg kg^{-1}, i.p. These effects are CB_1 receptor mediated and have been observed at doses of WIN55,212-2 that cause no significant alteration in the sensory thresholds of the

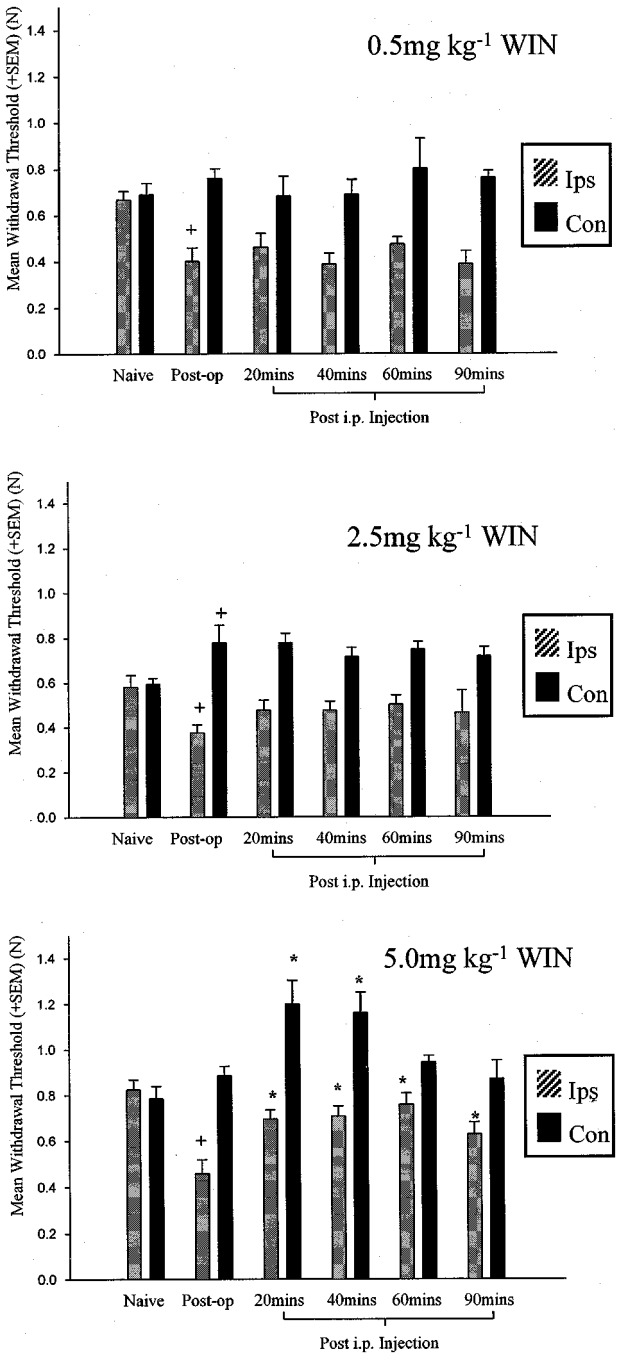

Figure 3 Bilateral hind limb withdrawal responses to mechanical stimulation (electronic Von Frey) in rats rendered neuropathic by L5 spinal nerve ligation administered WIN55,212-2 (0.5–5.0 mg kg^{-1}, i.p., $n=6$ per dose). Attenuation of mechanical allodynia was observed at a dose of 5.0 mg kg^{-1} WIN55,212-2. $+P<0.05$, paired *t*-test, $*P<0.05$, one-way ANOVA (Dunnett's).

uninjured contralateral hind limb. Neither administration of SR141716a alone, administration of solvent nor sham surgery had an effect on the sensory thresholds. These results concur with a previous study which examined the analgesic actions of WIN55,212-2 in the CCI model of neuropathic pain (Herzberg *et al*., 1997) despite important differences between the models.

The blockade of the anti-hyperalgesic and anti-allodynic effects of WIN55,212-2 by the CB_1 receptor antagonist, but not the CB_2 receptor antagonist demonstrates that the analgesic effect of WIN55,212-2 is mediated through the CB_1 receptor. This is despite the fact that WIN55,212-2, like most other cannabinoids, has significant affinity for the CB_2 receptor, as well as CB_1. The CB_1 receptor is localized throughout the central nervous system, and has been mapped by immunocytochemistry in the brain (Tsou *et al*., 1998), spinal cord (Farquhar-Smith *et al*., 2000) and in cultured dorsal root ganglion cells (Ahluwalia *et al*., 2000). Immunocytochemical study of the spinal cord has shown that the expression of CB_1 receptors is localized to areas associated with nociception, for example the superficial dorsal horn and lamina X (Farquhar-Smith *et al*., 2000). The localization of CB_1 receptors in lamina I and lamina II_i/III coincides with the terminals of some of the neurons lost in peripheral nerve injury. It would therefore be expected that CB_1 receptor expression would also decrease after peripheral nerve injury. However, Farquhar-Smith *et al*. (2000) observed no biologically relevant decrease in CB_1 receptor after dorsal rhizotomy or rostral hemisection of the spinal cord. The authors postulate that this finding could be explained by the majority of spinal CB_1 receptors being localized on spinal interneurons. This concept is supported by electrophysiological studies (Jennings *et al*., 2000) and changes in spinal cord receptor binding of [^{3}H]-CP55,940 after neonatal capsaicin treatment (Hohmann & Herkenham, 1998), where the authors found only a 16% reduction in CB_1 receptor binding after death of small diameter afferents was caused by capsaicin treatment. However, a multi-level dorsal rhizotomy (C3–T2) caused a 46% decrease in [^{3}H]-CP55,940 binding, suggesting a presence of CB_1 receptors on central termini of primary afferent neurons (Hohmann *et al*., 1999), although this finding could be explained by post-synaptic degeneration of neurons after such an extensive surgery.

A possible confounding factor in our experiments is a putative effect of WIN55,212-2 on the sensory thresholds *per se*. However, we have controlled for such an effect by measuring the sensory thresholds in the contralateral hind paw in all experiments. Only at the highest used dose of WIN55,212-2 was there a significant increase in the sensory threshold of the contralateral paw, suggesting that such an effect is not a confusing factor to the results at doses of WIN55,212-2 of 2.5 mg kg^{-1} or less.

The well-known psychotropic effects of cannabinoids are an obstacle to the development of cannabinoid based analgesics for the treatment of neuropathic pain (Perez-Reyes, 1999). Nevertheless, although not formally measured, we did not observe any obvious abnormal rodent behaviour indicative of psychotropic effects at the doses of WIN55,212-2 studies used in these experiments. However, there are a number of ways in which this issue may be circumvented: for example, structural engineering of cannabinoid molecules may permit certain cannabinoids to retain therapeutic activity whilst being devoid of psychotropic effects. One example of a cannabinoid which retains analgesic activity, in a model of inflammatory arthritis, in the absence of psychotropic effects is cannabidiol (Malfait *et al*., 2000). Furthermore, in a recent publication, Fox and colleagues have demonstrated that peripherally or spinal intrathecally administered WIN55,212-2, at doses that were not systemically active, attenuated the

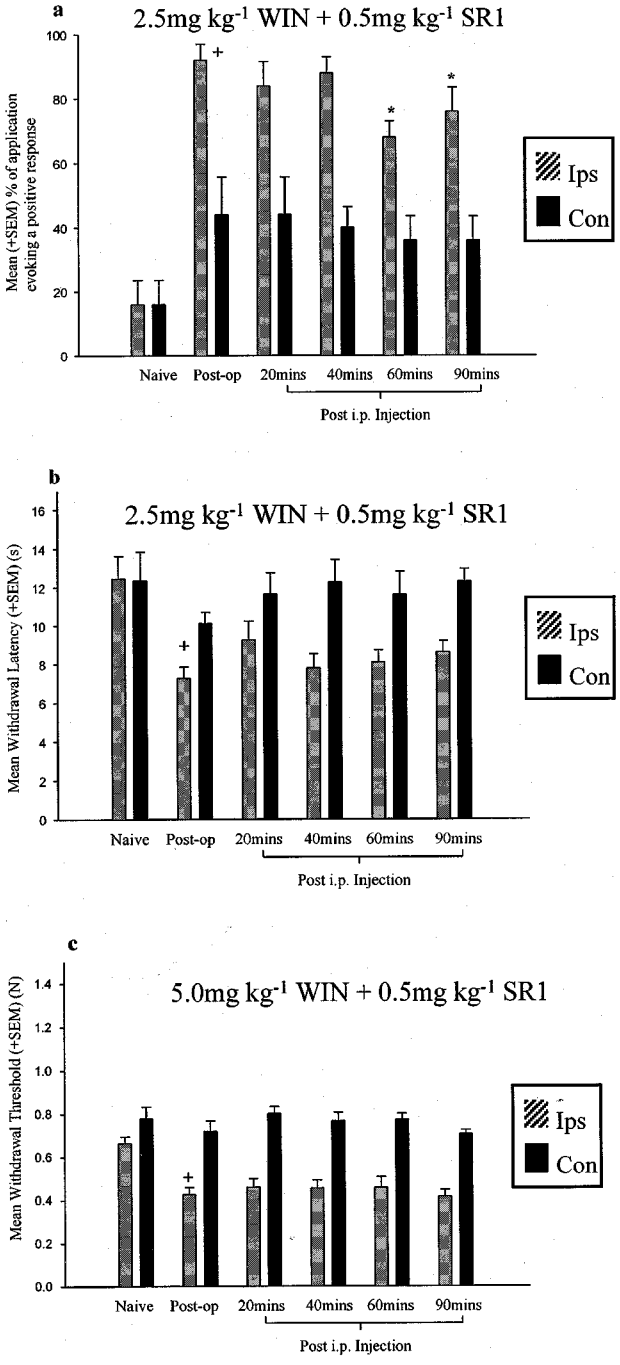

Figure 4 Bilateral hind limb withdrawal responses to (a) cold (acetone drop), (b) thermal (Hargreaves' device) and (c) mechanical (electronic Von Frey) stimulation in rats rendered neuropathic by L5 spinal nerve ligation ($n=6$ per group) co-administered SR141716a (0.5 mg kg^{-1}, i.p.) and an effective dose of WIN55,212-2. SR141716a reversed the anti-allodynic and anti-hyperalgesic effects of WIN55,212-2. $+P<0.05$, paired t-test, $*P<0.05$, one way ANOVA (Dunnett's).

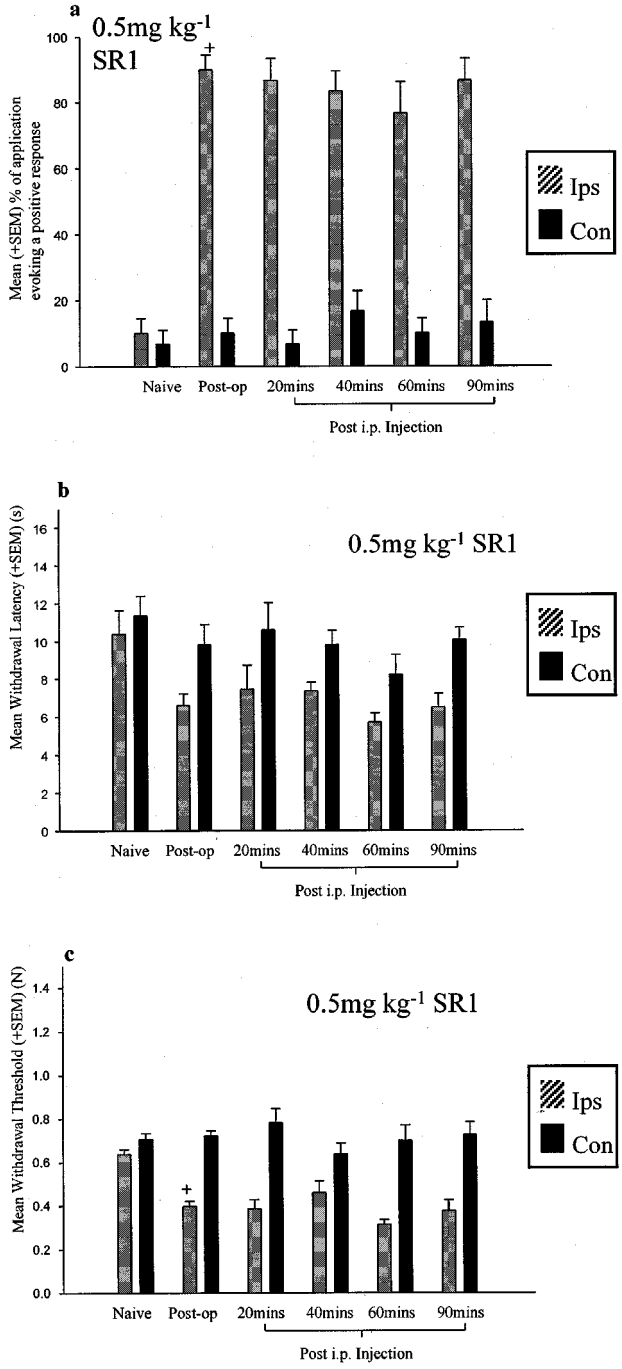

Figure 5 Bilateral hind limb withdrawal responses to (a) cold (acetone drop), (b) thermal (Hargreaves' device) and (c) mechanical (electronic Von Frey) stimulation in rats rendered neuropathic by L5 spinal nerve ligation ($n=6$ per group) administered SR141716a (0.5 mg kg^{-1}, i.p.) alone. SR141716a administered alone was associated with no change in sensory thresholds. $+P<0.05$, paired t-test.

altered sensory thresholds associated with partial sciatic nerve ligation (Fox *et al*., 2001). This suggests that either selective delivery of cannabinoids by these routes, or systemic administration of cannabinoids that do not penetrate into the CSF, may be a method of divorcing analgesic from psychotropic effects. Finally, Baker *et al*. (2001) have demonstrated, using a model of multiple sclerosis, that concentrations of endocannabinoids are selectively elevated in areas of neuronal injury in spinal cord and brain. This finding indicates that it may be possible to selectively manipulate endocannabinoid concentrations in areas of neuronal injury, by inhibiting their degradation.

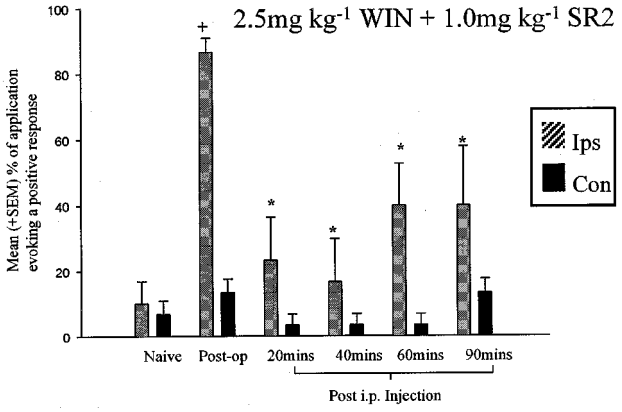

Figure 6 Bilateral hind limb withdrawal responses to cold (acetone drop) stimulation in rats rendered neuropathic by L5 spinal nerve ligation ($n=6$) co-administered SR144528 (1.0 mg kg^{-1}, i.p.) and WIN55,212-2 (2.5 mg kg^{-1}, i.p.). SR144528 did not attenuate the anti-allodynic effect of WIN55,212-2. $+P<0.05$, paired t-test), $*P<0.05$, one way ANOVA (Dunnett's).

It has also been demonstrated that [^{3}H]-DAMGO (a μ-opioid receptor agonist) binding was reduced by 60% following neonatal capsaicin treatment (Hohmann & Herkenham, 1998) and by 62% following multi-level dorsal rhizotomy (Hohmann *et al*., 1999). This consistent loss of opioid receptors following nerve injury is a factor for the relative ineffectiveness of morphine in neuropathic pain. Also, with several studies documenting a stable population of CB_1 receptors in the superficial dorsal horn after peripheral nerve injury, a potential therapeutic advantage for cannabinoids over opioids has been found at the level of the spinal cord. This hypothesis is further supported by a study in which it was observed that THC alleviated hyperalgesia in the CCI model of neuropathic pain (Mao *et al*., 2000). This effect was shown to be on CB_1 and not opioid receptor dependent, with no cross tolerance between opioids and cannabinoids, suggesting a different pathway of antinociception for cannabinoids and opioids.

In this study, we were unable to replicate the increase in allodynia or hyperalgesia associated with administration of the CB_1 receptor antagonist SR141716a alone to animals with peripheral nerve injury that was observed by Herzberg *et al*. (1997). In our study, there was no significant alteration in sensory thresholds in SR141716a treated animals when compared to solvent treated controls. The increase of hyperalgesia or allodynia seen after administration of the receptor antagonist has been hypothesized to suggest the presence of an endogenous tone that is active during neuropathy – viz. locally synthesized endocannabinoids act at a low level on the receptors in response to peripheral nerve injury, causing a degree of attenuation of the hyperalgesia or allodynia. Thus, by blocking the action of these endogenous ligands by administration of the specific antagonist, the hyperalgesia or allodynia will be increased. The current data on an endogenous cannabinoid tone in pain models is controversial. Several studies have shown the presence of a SR141716a-associated enhancement of signs of pain or nociception in a variety of models, including the formalin test for inflammation (Calignano *et al*., 1998; Strangman *et al*., 1998). This could be interpreted as supporting the hypothesis that an endogenous tone exists in inflammatory pain, but not in neuropathic pain. If this is the case, then the inflammatory component of the CCI model could account for the difference seen between this and the findings of Herzberg *et al*. (1997). However, other studies of endogenous tone in inflammatory models have found no evidence supporting this concept, either in an inflammatory model (Beaulieu *et al*., 2000) or when administered to uninjured animals (Rinaldi-Carmona *et al*., 1995). The use of SR141716a as an experimental tool to reveal endogenous tone could be further confused by the findings of several groups that SR141716a is actually an inverse agonist at the CB_1 receptor (Landsman *et al*., 1997; Pan *et al*., 1998). Further studies in $CB_1-/-$ knockout mice did not support the concept of an endogenous tone (Ledent *et al*., 1999). It also remains to be determined whether the levels of endogenous cannabinoids found in the spinal cord and dorsal root ganglia are sufficient to activate the receptors. Despite the controversial nature of the endogenous tone, our experiments seem to contradict its existence and agree with the study by Beaulieu *et al*. (2000) that there is a weak analgesic action at about 60 min post-administration, possibly due to SR141716a being able to show weak agonist activity under certain conditions.

This study has demonstrated the existence of CB_1 receptor, but not CB_2 receptor, mediated analgesia in an animal model of neuropathic pain. In light of the current therapeutic need for neuropathic pain treatments, this study provides evidence for the potential of cannabinoids, or inhibitors of degradation of endocannabinoids, as lead compounds for development of new therapies. The development of cannabinoid receptor specific agonists and antagonists will provide essential new tools for investigating the mechanism of this analgesic action.

D. Bridges is funded by an industrial collaborative grant from the MRC awarded to Novartis Institute of Medical Sciences. The authors are grateful to Dr J. Winter and Dr W.P. Farquhar-Smith for helpful discussions and to Dr J.W. Brooks for randomization. Part of this study has been previously presented at the 2000 Symposium on Cannabinoids, International Cannabinoid Research Society, 22/6/00, Baltimore, MD, U.S.A.

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(Received January 29, 2001
Revised April 9, 2001
Accepted April 9, 2001)