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# The role of central and peripheral Cannabinoid$_1$ receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain

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**Abstract**

We have examined the effects of cannabinoid agonists on hyperalgesia in a model of neuropathic pain in the rat and investigated the possible sites of action. The antihyperalgesic activity of the cannabinoids was compared with their ability to elicit behavioural effects characteristic of central cannabinoid activity. WIN55,212-2 (0.3–10 mg kg$^{-1}$), CP-55,940 (0.03–1 mg kg$^{-1}$) and HU-210 (0.001–0.03 mg kg$^{-1}$) were all active in a 'tetrad' of tests consisting of tail-flick, catalepsy, rotarod and hypothermia following subcutaneous administration, with a rank order of potency in each of HU-210 > CP-55,940 > WIN55,212-2. The effects of WIN55,212-2 in each assay were blocked by the Cannabinoid$_1$ ($CB_1$) antagonist SR141716A. In the partial sciatic ligation model of neuropathic pain WIN55,212-2, CP-55,940 and HU-210 produced complete reversal of mechanical hyperalgesia within 3 h of subcutaneous administration with $D_{50}$ values of 0.52, 0.08 and 0.005 mg kg$^{-1}$, respectively. In this model WIN55,212-2 was also effective against thermal hyperalgesia and mechanical allodynia. WIN55,212-2 produced pronounced reversal of mechanical hyperalgesia following intrathecal administration that was blocked by the $CB_1$ antagonist SR141716A. Following intraplantar administration into the ipsilateral hindpaw, WIN55,212-2 produced up to 70% reversal of mechanical hyperalgesia, although activity was also observed at high doses following injection into the contralateral paw. The antihyperalgesic effect of WIN55,212-2 injected into the ipsilateral paw was blocked by subcutaneously administered SR141716A, but was not affected by intrathecally administered SR141716A. These data show that cannabinoids are highly potent and efficacious antihyperalgesic agents in a model of neuropathic pain. This activity is likely to be mediated via an action in both the CNS and in the periphery. © 2001 International Association for the Study of Pain.

*Keywords*: Central and peripheral Cannabinoid$_1$ receptors; Antihyperalgesic activity of cannabinoids; Model of neuropathic pain

## 1. Introduction

There is now considerable evidence supporting a role for cannabinoids in nociception. Cannabinoid$_1$ ($CB_1$) receptors are expressed in key areas involved in nociception, including the periaqueductal grey, the dorsal horn of the spinal cord and dorsal root ganglion neurones (Tsou et al., 1998; Hohmann and Herkenham, 1999a,b), and a wide range of behavioural studies have shown that cannabinoids are antinociceptive in animal models of acute pain. Thus, the naturally occurring $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) and synthetic cannabinoids such as WIN55,212-2 and CP-55,940 inhibit responses to noxious thermal and mechanical stimuli in the hot-plate, tail-flick and paw pressure tests (Sofia et al., 1973; Lichtman and Martin, 1991; Welch et al., 1998; Martin et al., 1996; Smith et al., 1998), as well as nociceptive behaviours in the formalin test (Moss and Johnson, 1980; Tsou et al., 1996). The endogenous $CB_1$ receptor ligand anandamide is similarly antinociceptive in these tests (Fride and Mechoulam, 1993; Smith et al., 1994; Calignano et al., 1998). More recently, it has been shown that WIN55,212-2 and anandamide are also effective against more persistent nociceptive processes, reducing thermal and mechanical hyperalgesia and mechanical allodynia following peripheral inflammation (Richardson et al., 1998a,b; Martin et al., 1999), capsaicin injection (Li et al., 1999) or peripheral nerve injury (Herzberg et al., 1997). In addition, the $\Delta^9$-THC analogue HU-2111 has been shown to reduce autotomy induced by peripheral nerve section (Zeltser et al., 1991). Both the analgesic and antihyperalgesic effects of cannabinoids are mediated by activation of $CB_1$ receptors and are blocked by the selective antagonist SR141716A (Rinaldi-Carmona et al., 1994; Richardson et al., 1998a; Calignano et al., 1998; Lichtman and Martin, 1997).

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The analgesic and antihyperalgesic activity of cannabinoids is generally held to be mediated via a central site of action. This conclusion is supported by the antinociceptive activity of cannabinoids following intrathecal or intracerebroventricular administration (Yaksh, 1981; Martin et al., 1993, 1995, 1999; Richardson et al., 1998b; Welch et al., 1998). Electrophysiological studies have shown that WIN55,212-2 inhibits responses of thalamic and spinal dorsal horn neurones to acute peripheral noxious thermal or mechanical stimuli (Martin et al., 1996; Hohmann et al., 1998, 1999) as well as the wind-up response to prolonged noxious stimulation (Strangman and Walker, 1999). WIN55,212-2 similarly inhibits the formalin-induced expression of c-fos in the spinal cord and thalamus (Tsou et al., 1996; Martin et al., 1996) and capsaicin-induced peptide release in the spinal cord (Richardson et al., 1998b). However, there is also evidence that cannabinoids may modulate nociceptive processes via an action at peripheral receptors. Thus, peripheral intraplantar, but not systemic, administration of anandamide inhibits carrageenan-induced hyperalgesia and oedema, as well as capsaicin-induced oedema and peptide release in the hindpaw (Richardson et al., 1998a). A similar inhibition of the behavioural response to formalin injection was observed following intraplantar injection of anandamide and the putative endogenous $CB_2$ receptor ligand palmitoylethanolamide (PEA; Calignano et al., 1998).

$CB_1$ receptors are distributed throughout the CNS and cannabinoids have long been known to produce a variety of centrally-mediated effects in addition to their antinociceptive activity. In man there are obvious psychotropic effects, and administration to animals elicits catalepsy, deficits in motor performance and hypothermia, which together with acute antinociception form the 'tetrad' of tests for cannabinoids in mice (Compton et al., 1992; Smith et al., 1994). Such side effects limit the potential clinical use of cannabinoids for analgesia, and could confound antinociceptive or antihyperalgesic measurements in animal studies which generally rely on paw or tail withdrawal from a noxious stimulus. There are many studies examining the effects of cannabinoids on acute pain and more chronic inflammatory pain, but much less is known of their effects on neuropathic pain. Here we have examined the effects of three standard synthetic cannabinoids, WIN55,212-2, CP-55,940 and HU-210, in a model of neuropathic pain in the rat and provide evidence for a peripheral site of action in this model. Moreover, we provide a full comparison of the antihyperalgesic activities of these ligands with their activities in the tetrad models in the rat.

## 2. Methods

### 2.1. Neuropathic pain model

Hyperalgesia and allodynia were examined in the model of neuropathic pain induced by partial ligation of the sciatic nerve as described by Seltzer et al. (1990). Briefly, Wistar rats (120–140 g) were anaesthetized, the left sciatic nerve was exposed at mid-thigh level through a small incision and 1/3 to 1/2 of the nerve thickness was tightly ligated within a 7.0 silk suture. The wound was closed with a single muscle suture and skin clips and dusted with Aureomycin antibiotic powder. The animals were allowed to recover and used 12–15 days following surgery.

#### 2.1.1. Mechanical hyperalgesia

Mechanical hyperalgesia was assessed by measuring paw withdrawal thresholds to an increasing pressure stimulus placed onto the dorsal surface of the paw using an analgesymeter (Ugo Basile, Milan) with a cut-off of 250 g. Withdrawal thresholds were measured on both the ipsilateral (ligated) and contralateral (unligated) paw prior to (predose) and then up to 6 h following drug or vehicle administration. We have previously determined that partial nerve ligation does not affect contralateral withdrawal thresholds, and sham surgery does not affect ipsilateral thresholds. Reversal of hyperalgesia at each time point was therefore calculated according to the following formula, which uses the contralateral paw as a reference rather than using additional groups of naive or sham animals:

$$\%\ \text{reversal} = \frac{\text{ipsilateral threshold postdose} - \text{ipsilateral threshold predose}}{\text{contralateral threshold predose} - \text{ipsilateral threshold predose}} \times 100$$

#### 2.1.2. Thermal hyperalgesia

Thermal hyperalgesia was examined by measuring the latency to withdrawal of the hindpaws from a focussed beam of radiant heat applied to the plantar surface using a Ugo Basile Plantar Test apparatus. Two days prior to the experiment animals were placed in a transparent Perspex box with a thin glass floor and allowed to acclimatize for 10–15 min before withdrawal latencies were measured on both ipsilateral and contralateral paws. On the day of the experiment withdrawal latencies were measured immediately prior to and then 3 h following subcutaneous administration of WIN55,212-2 or vehicle. An additional group of age-matched naive animals was included as a positive control. On all three occasions the withdrawal latency of each paw was measured twice with a 5 min interval and the mean of the two readings was used for data analysis. With each reading the apparatus was set with a cut-off time of 32 s.

#### 2.1.3. Tactile allodynia

Tactile allodynia was assessed by measuring withdrawal thresholds to calibrated von Frey hairs with intensities ranging from 1 to 20.9 g. Filaments exerting a force above 20.9 g were not used as they produced lifting of the paws. On the day of the experiment animals were placed in a

Perspex chamber with a mesh metal floor and allowed to acclimatize for 15–30 min. Starting with the lowest filament force, von Frey hairs were applied perpendicular to the mid plantar surface of both hind paws, with sufficient force to cause slight bending against the paw, and held for a few seconds. This was repeated five times with an interval of 1–2 s. A positive response was noted if the paw was sharply withdrawn or if there was flinching upon removal of the hair. If no response was noted to any trial the process was repeated with the next higher force hair and the filament which produced a positive response was denoted as the threshold. At least 5 min was left between trials with successive filaments. Withdrawal thresholds were determined prior to and 3 h following administration of WIN55,212-2 (0.3–3 mg $kg^{-1}$) or vehicle. An additional group of age-matched naive animals was included as a positive control.

### 2.2. Tetrad

#### 2.2.1. Tail-flick

Antinociceptive activities of test compounds were assessed using the tail-flick assay. Animals were placed inside a cotton pouch and the tail was exposed to a focused beam of radiant heat at a point 3 cm from the tip using a tail-flick unit (Ugo Basile, Italy). An infrared intensity of 14 was used in all experiments. Tail-flick latencies were defined as the interval between the onset of the thermal stimulus and withdrawal of the tail. A 15 s cut-off was employed to prevent tissue damage. Withdrawal latencies were measured immediately prior to (predose) and then up to 6 h following drug or vehicle administration. Data are expressed as the latency (s) and percentage of the maximum possible effect (% MPE) as defined below:

$$\%\ \text{MPE} = \left(\frac{\text{postdrug latency} - \text{predrug latency}}{\text{cut-off (15 s)} - \text{predrug latency}}\right) \times 100$$

#### 2.2.2. Rotarod

Changes in motor performance were assessed using the accelerating rotarod (Ugo Basile, Italy) in which rats were required to walk against the motion of a rotating drum, with the speed increasing from 4 to 40 rev./min over 5 min. The time taken to fall off the rotarod was recorded as the latency (s). Twenty-four hours prior to drug testing rats were given two training periods on the rotarod 1–2 h apart until they were able to remain on the rotarod for at least 60 s. During drug testing rotarod latencies were measured immediately prior to and up to 6 h following drug or vehicle administration. In all experiments a 300 s cut-off was employed. Data are expressed as the latency (s), and the percentage disruption in performance was calculated as:

$$100 - (\text{[postdrug latency/predrug latency]} \times 100)$$

#### 2.2.3. Catalepsy

Catalepsy was measured using a modified 'ring test' as originally described by Pertwee (1972). Rats were hung by their front paws from a rubber coated metal ring (12 cm diameter) fixed horizontally at a height allowing their hind-paws to just touch the bench, and the time taken for the rat to move off the ring was measured with a cut-off of 30 s. Latencies were measured immediately prior to and up to 6 h following drug or vehicle administration. Data are expressed as the latency (s) and percentage of maximum possible effect (% MPE) as defined below:

$$\%\ \text{MPE} = \left(\frac{\text{postdrug latency} - \text{predrug latency}}{\text{cut-off (30 s)} - \text{predrug latency}}\right) \times 100$$

#### 2.2.4. Hypothermia

Core temperatures in the rat were measured using a BAT-12 thermometer and a lubricated RET-2 rectal probe (Sensortek, USA) inserted into the rectum to a constant depth of 2.5 cm. The minimum effective dose is defined as the lowest dose giving a statistically significant change in temperature from time-matched vehicle-treated controls using Tukey's HSD test ($P < 0.05$ was considered significant).

### 2.3. Drugs

WIN55,212-2, CP-55,940 and HU-210 were obtained from Tocris Cookson (UK) and made up in (v/v) 20% DMSO/1% ethanol/1% Tween-80/78% saline. Anandamide (Tocris) was supplied at a stock concentration of 10.1 mg $ml^{-1}$ in soya oil/water (1:4) emulsion and then diluted in saline as required. SR141716A was obtained from and dissolved in 20% cremaphor/saline. Drugs were administered subcutaneously in a volume of 0.5 ml or into the intraplantar region of the hindpaw in a volume of 10 μl. Intrathecal injections were carried out essentially as described previously (Mestre et al., 1994). Under light enflurane anaesthesia animals were held by the pelvic girdle and a $26^{3}/_{8}$ G needle attached to a 50 μl Hamilton syringe was inserted into the tissues between the dorsal aspects of the L4 and L5 spinal processes. Injection volumes of 10 μl were used.

### 2.4. Data analysis

Statistical analysis for behavioural experiments was carried out on paw withdrawal thresholds or latency measurements using ANOVA followed by Tukey's HSD test comparing drug-treated with vehicle-treated animals. $D_{50}$ values, representing the dose required to produce a 50% reversal of predose mechanical hyperalgesia, are quoted for the hyperalgesia assay as frequently maximal drug activity is not reached and there is no maximum possible effect to the assay. $ED_{50}$ values have been quoted for the tetrad tests, where a maximum response is imposed, and represent 50% MPE in the tail-flick and catalepsy tests, and 50% disruption in rotarod performance.

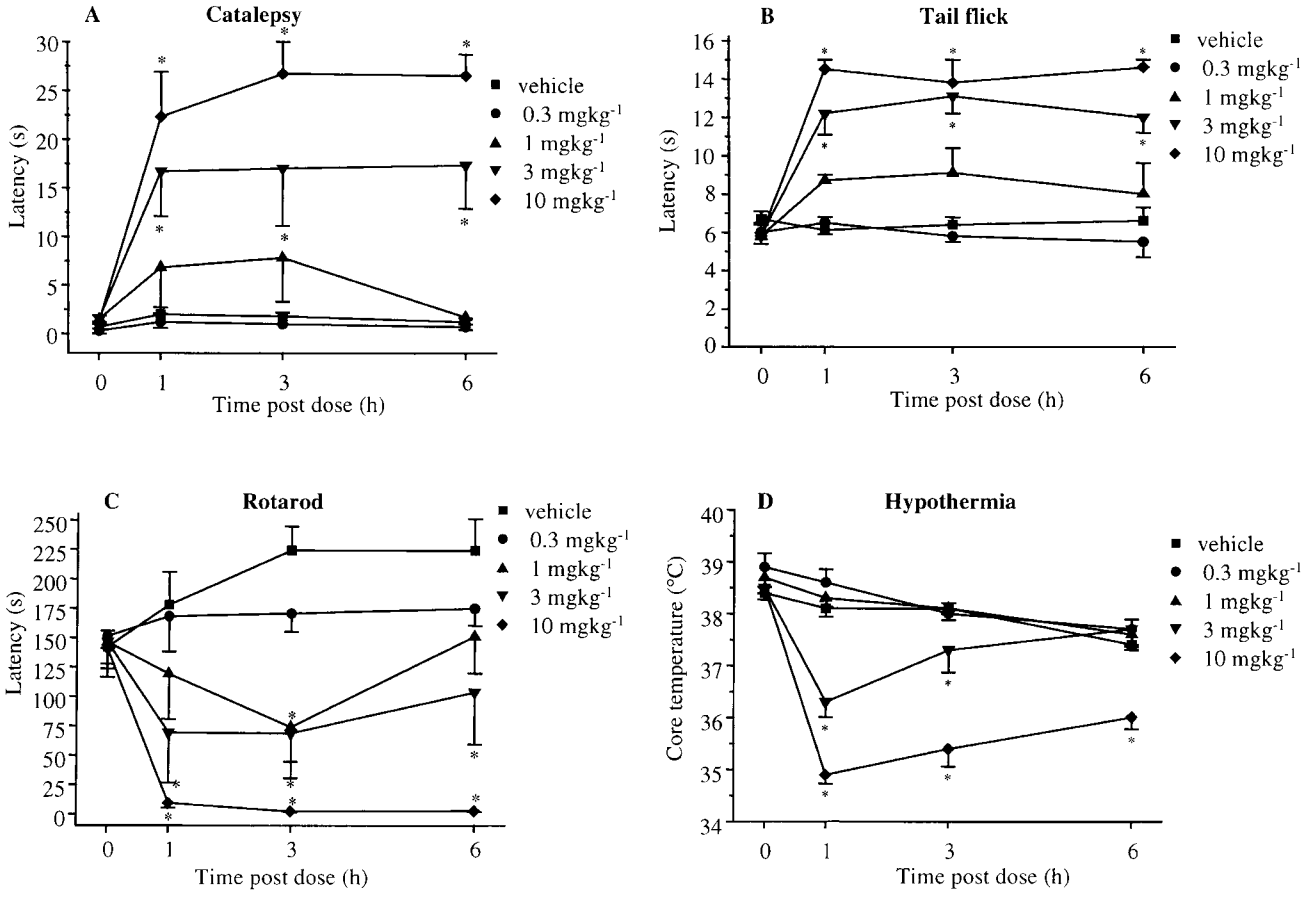


Fig. 1. Activity of WIN55,212-2 in the (A) catalepsy, (B) rotarod, (C) tail-flick and (D) hypothermia tests. Animals were tested in each assay prior to (0 h) and up to 6 h after subcutaneous administration of WIN55,212-2 or vehicle. (A–D) Each point represents the mean ± SEM from six animals per treatment group. *$P < 0.05$ compared to vehicle by ANOVA plus Tukey's HSD test.

## 3. Results

### 3.1. Tetrad

WIN55,212-2, CP-55,940 and HU-210 were all active in the classical 'tetrad' of tests for cannabimimetic agents following subcutaneous administration. Thus, the three compounds elicited catalepsy, motor dysfunction as seen using the rotarod assay, antinociception in the tail-flick assay and hypothermia. Dose–response curves for WIN55,212-2 in each of these tests are shown in Fig. 1. Similar profiles were observed for CP-55,940 and HU-210 with effects in each assay apparent within 1 h of administration and lasting for at least 6 h. $ED_{50}$ values for each compound in these assays are shown in Table 1 from which it can be seen that in each test there is a rank order of potency of HU-210 > CP-55,940 > WIN55,212-2. Administration of the selective $CB_1$ receptor antagonist SR141716A (1 mg $kg^{-1}$, s.c.) 30 min prior to administration of $ED_{50}$ doses of WIN55,212-2 inhibited the effects of the cannabinoid agonist in each assay (Fig. 2). At the dose used the antagonist alone did not produce significant effects in any of the tetrad assays.

### 3.2. Neuropathic hyperalgesia

Following partial sciatic nerve ligation, ipsilateral paws exhibited pronounced mechanical hyperalgesia with predose

Table 1
Comparison of the activities of cannabinoids against neuropathic mechanical hyperalgesia and the tetrad of tests for CNS activity[a]

| Assay | $D_{50}/ED_{50}$ (mg $kg^{-1}$, s.c.) | | |
|---|---|---|---|
| | WIN55,212-2 | CP-55,940 | HU-210 |
| Hyperalgesia | 0.52 | 0.08 | 0.005 |
| Rotarod | 0.87 | 0.27 | 0.023 |
| Tail-flick | 1.44 | 0.061 | 0.013 |
| Catalepsy | 2.61 | 0.52 | 0.017 |
| Hypothermia | 3 | 0.3 | 0.03 |

[a] Data shown are $D_{50}$ or $ED_{50}$ values obtained 3 h following subcutaneous administration in each assay. $D_{50}$ values, representing the dose required to produce 50% reversal of predose hyperalgesia, are calculated for the hyperalgesia assay, and $ED_{50}$ values are calculated for the tetrad tests, representing the dose producing 50% disruption of rotarod performance, and 50% MPE in the tail-flick and catalepsy tests (see Section 2). Values for hypothermia represent the lowest dose producing a significant change in core temperature.

withdrawal thresholds averaging approximately 65 g compared to contralateral withdrawal thresholds of approximately 110 g in all experiments. Subcutaneous administration of WIN55,212-2, CP-55,940 or HU-210 produced pronounced reversal of established hyperalgesia that was apparent within 1 h of administration and lasted for at least 6 h (Fig. 3). All were highly efficacious with a maximal 100 ± 11% reversal of hyperalgesia produced by WIN55,212-2, 144 ± 13% reversal by CP-55,940, and 103 ± 9% reversal by HU-210. Of the three compounds, HU-210 was approximately 100 times more potent than WIN55,212-2 and 15 times more potent than CP-55,940 (Table 1). WIN55,212-2 produced a slight increase in the contralateral paw withdrawal threshold of approximately 10 g only at the highest dose tested (3 mg $kg^{-1}$). More pronounced contralateral effects were seen with CP-55,940 and HU-210 which both produced increases of approximately 20 g at the highest doses (0.3 and 0.03 mg $kg^{-1}$, respectively). All three compounds additionally produced observable side effects at the higher doses including sedation, urination and catalepsy. Further experiments were carried out with WIN55,212-2 as this compound generally produced the least severe side effects.

The possible site of action of cannabinoids in this model was investigated by administration of WIN55,212-2 at peripheral and spinal sites. Intrathecal administration of WIN55,212-2 (1–100 μg) produced a marked reversal of mechanical hyperalgesia with a maximal 63 ± 8% reversal observed 1 h following administration (Fig. 4A). At the highest dose (100 μg), there were also effects on the contralateral paw with an increase in withdrawal thresholds of up to 20 g. The antihyperalgesic effect of intrathecally administered WIN55,212-2 (30 μg) was inhibited by co-administration of SR141716A (30 μg). At this dose WIN55,212-2 did not affect contralateral paw withdrawal thresholds, and the antagonist alone did not affect either ipsilateral or contralateral withdrawal thresholds (Fig. 4B).

Peripheral administration of WIN55,212-2 or anandamide produced a rapid reversal of mechanical hyperalgesia. Thus, intraplantar injection of 30 and 100 μg WIN55,212-2 into the ipsilateral hindpaw produced a maximal 68 ± 4% reversal 15 min following administration (Fig. 5). The anti-

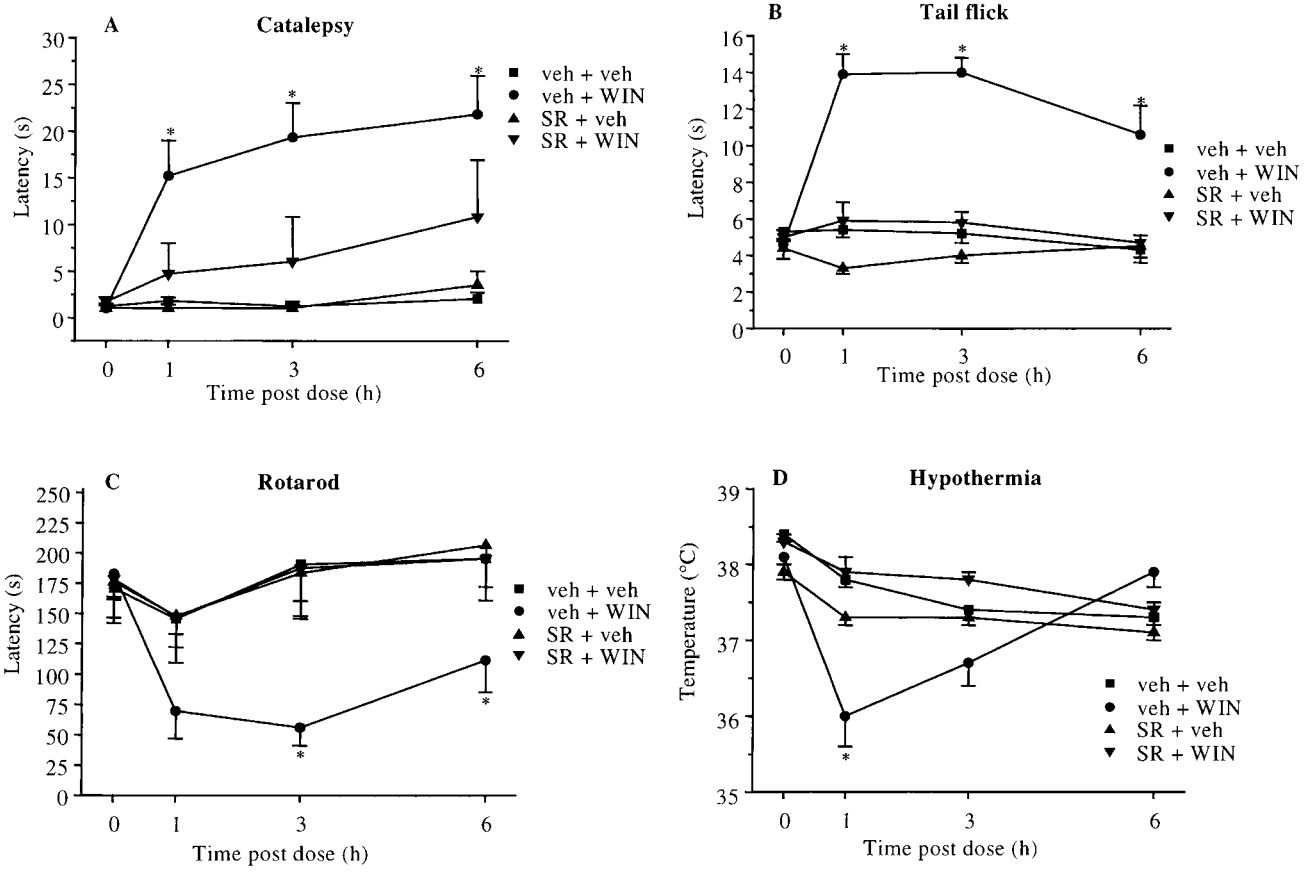


Fig. 2. Inhibition by SR141716A of the effects of WIN55,212-2 in the (A) catalepsy, (B) rotarod, (C) tail-flick and (D) hypothermia tests. Animals were tested in each assay prior to (0 h) and then up to 6 h following subcutaneous administration of WIN55,212-2 (WIN, 1.5 mg $kg^{-1}$ for tail-flick and hypothermia, 3 mg $kg^{-1}$ for catalepsy and rotarod) or vehicle (veh). SR141716A (SR, 1 mg $kg^{-1}$) or vehicle was administered subcutaneously 30 min prior to WIN55,212-2 administration. (A–D) Each point represents the mean ± SEM from six animals per treatment group. *$P < 0.05$ compared to vehicle by ANOVA plus Tukey's HSD test.

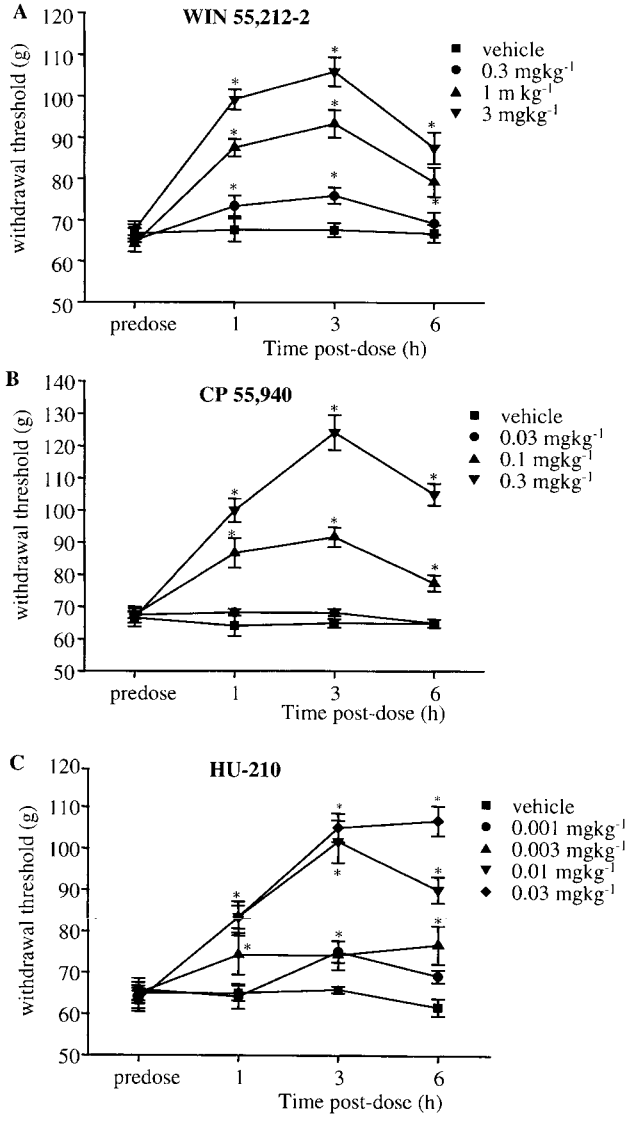


Fig. 3. The effect of subcutaneous administration of (A) WIN55,212-2, (B) CP-55,940 and (C) HU-210 on neuropathic mechanical hyperalgesia. Paw withdrawal thresholds were measured prior to and up to 6 h following drug or vehicle administration. (A–C) Each point represents the mean ± SEM paw withdrawal threshold from the ipsilateral paw from six animals per treatment group. *$P < 0.05$ compared to vehicle treatment by ANOVA followed by Tukey's HSD test.

hyperalgesic effect was long lasting with significant activity still apparent 3 h following administration. To test whether this effect of WIN55,212-2 represented local activity within the paw, or was due to systemic absorption following the intraplantar injection, the effects of WIN55,212-2 on the ipsilateral hyperalgesia were also examined in the same experiment following intraplantar injection into the contralateral paw. As can be seen from Fig. 5, injection of 100 μg into the contralateral paw produced a similar reversal of hyperalgesia to that seen following ipsilateral injection. However, contralateral injection of 30 μg produced a maximal 24 ± 6% reversal which was significantly less than the activity observed following ipsilateral injection of 30 μg (50 ± 3.1% reversal). The antihyperalgesic effect of WIN55,212-2 following intraplantar injection into the ipsilateral paw was blocked by subcutaneous administration of SR141716A (1 mg $kg^{-1}$, s.c.) (Fig. 6A) but was not affected by intrathecal administration of SR141716A at a dose (30 μg) previously found to inhibit the antihyperalgesic effect of intrathecally administered WIN55,212-2 (Fig. 6B).

### 3.3. Tactile allodynia

Following partial sciatic nerve ligation, animals also exhibited mechanical allodynia on the ipsilateral paw, whilst contralateral withdrawal thresholds were similar to those seen in naive animals. Subcutaneous administration of WIN55,212-2 (0.3–3 mg $kg^{-1}$, s.c.) produced a dose-related reversal of mechanical allodynia. At the highest dose used, von Frey hair thresholds were not significantly different from those observed in age-matched naive animals or contralateral paws of vehicle-treated animals (Fig. 7A). No increases in contralateral paw withdrawal thresholds

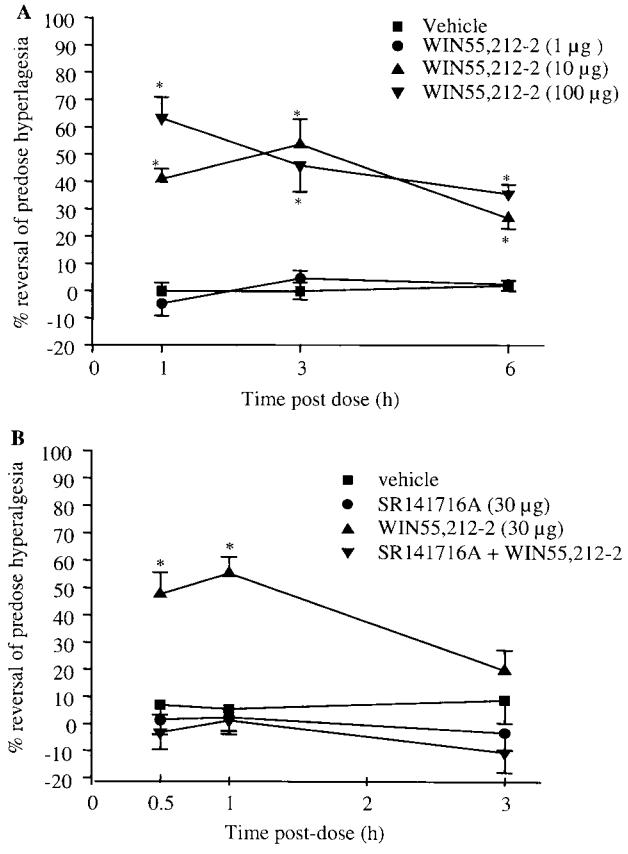


Fig. 4. The effect of intrathecal administration of WIN55,212-2 and SR141716A on neuropathic mechanical hyperalgesia. Paw withdrawal thresholds were measured prior to and 30 min, 1 h and 3 h following drug or vehicle administration and the data are presented as the percentage reversal of predose hyperalgesia. (A) Effect of WIN55,212-2 alone. (B) Antagonism of the antihyperalgesic effect of WIN55,212-2 by co-administration of SR141716A. (A,B) Each point represents the mean ± SEM from six animals per treatment group. *$P < 0.05$ compared to vehicle treatment by ANOVA followed by Tukey's HSD test carried out on g threshold data.

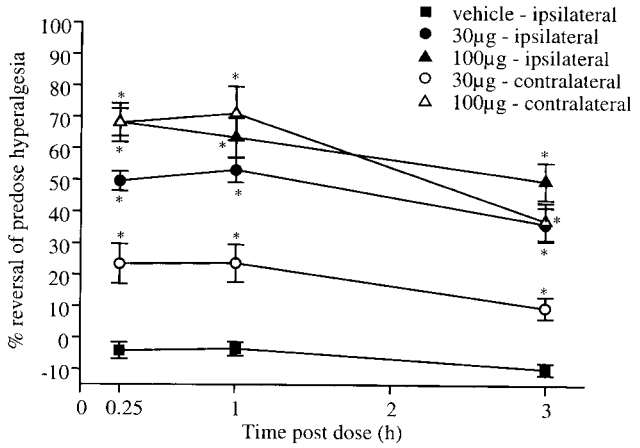


Fig. 5. The effect of intraplantar injection of WIN55,212-2 on neuropathic mechanical hyperalgesia. Paw withdrawal thresholds were measured prior to and 15 min, 1 h and 3 h following drug administration into either the ipsilateral or contralateral paw. Data are presented as the percentage reversal of predose hyperalgesia in the ipsilateral paw. Each point represents the mean ± SEM from six animals per treatment group. $^*P < 0.05$ compared to ipsilateral vehicle treatment by ANOVA followed by Tukey's HSD test carried out on g threshold data.

were observed, although it should be noted that the highest force exerted in these experiments was 20.9 g (see Section 2).

### 3.4. Thermal hyperalgesia

Thermal hyperalgesia was observed on the ipsilateral paw of ligated animals with a reduction in withdrawal latency of approximately 7 s compared to latencies measured in naive animals. Contralateral withdrawal latencies did not differ significantly from readings obtained in naive animals. WIN55,212-2 produced a significant increase in withdrawal latencies compared to predose readings only following administration of 3 mg $kg^{-1}$ (s.c.) (Fig. 7B). At this dose ipsilateral withdrawal latencies were increased above those measured in naive animals, and contralateral withdrawal latencies were also increased from 16.2 ± 2.1 to 27.2 ± 1.5 s. Lower doses did not affect contralateral readings.

## 4. Discussion

The acute antinociceptive effects of cannabinoids are well established (see Richardson, 2000, for review), but only recently has attention focussed on their effects in persistent pain states. A number of studies have shown that cannabinoids are antihyperalgesic in animal models of persistent inflammatory pain. Thus, anandamide has been shown to inhibit carrageenan-induced thermal hyperalgesia (Richardson et al., 1998a,b), and WIN55,212-2 inhibits complete Freund's adjuvant-induced mechanical allodynia (Martin et al., 1999). Additionally, anandamide, PEA and WIN55,212-2 all inhibit formalin-induced nociceptive behaviours (Calignano et al., 1998; Tsou et al., 1996; Jaggar et al., 1998). In contrast, much less is known of the effects of cannabinoids in neuropathic pain, with a single study showing that intraperitoneal administration of WIN55,212-2 inhibits mechanical and thermal hyperalgesia and tactile allodynia in the chronic constriction injury model of neuropathic pain (Herzberg et al., 1997). The present study provides an analysis of the activity of three synthetic cannabinoids in the partial sciatic ligation (Seltzer) model of neuropathic pain. WIN55,212-2, CP-55,940 and HU-210 were all potent and efficacious antihyperalgesic agents producing complete reversal of mechanical hyperalgesia. Additionally, WIN55, 212-2 reversed tactile allodynia and thermal hyperalgesia in this model. However, the effect on thermal hyperalgesia was

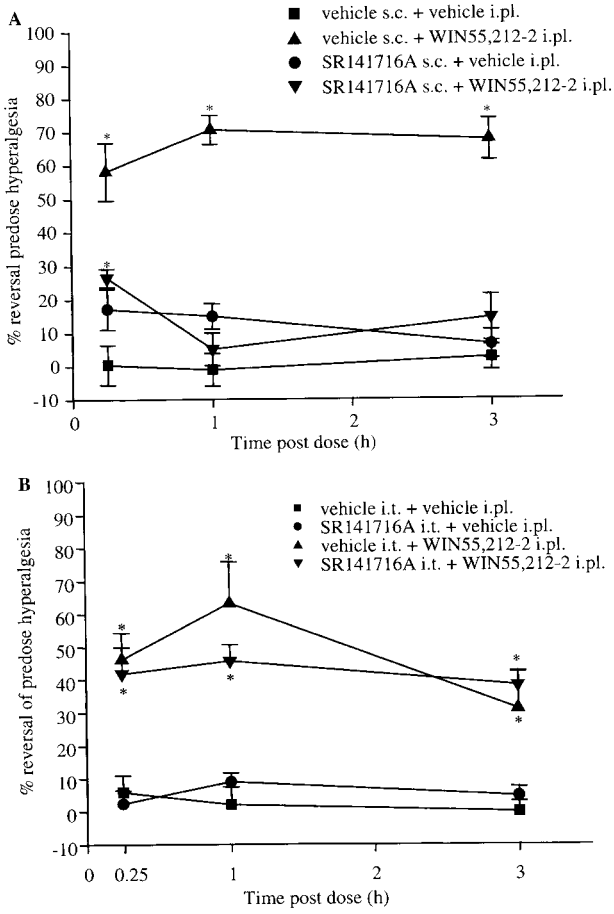


Fig. 6. The effect of SR141716A on the antihyperalgesic activity of WIN55,212-2 injected into the hindpaw. (A) Inhibition of the effect of intraplantar injection of WIN55,212-2 (100 μg) by subcutaneous administration of SR141716A (1 mg $kg^{-1}$). (B) Lack of effect of intrathecal administration of SR141716A (30 μg) on the antihyperalgesic activity of intraplantar administration of WIN55,212-2 (100 μg). (A,B) SR141716A was administered 30 min prior to WIN55,212-2. Paw withdrawal thresholds were measured prior to SR141716A administration and 15 min, 1 h and 3 h following administration of WIN55,212-2 into the ipsilateral paw, and data are presented as the percentage reversal of predose hyperalgesia. Each point represents the mean ± SEM from six animals per treatment group. $^*P < 0.05$ compared to vehicle treatment by ANOVA followed by Tukey's HSD test carried out on g threshold data.

significant only at a dose which also produced an increase in contralateral withdrawal latencies. Whilst this could indicate an analgesic effect, it could also result from the effects of WIN55,212-2 on motor performance as at this dose WIN55,212-2 was active in both the rotarod and catalepsy tests. It is interesting to note that WIN55,212-2 appears more potent against mechanical hyperalgesia and allodynia than thermal hyperalgesia. The reasons for this are unclear although it could indicate a differential role for $CB_1$ receptors in modulating the processing of responses to these different modalities, or a varying expression of $CB_1$ receptors on afferents encoding each modality.

In addition to their effects on nociceptive processing, cannabinoids produce other characteristic behavioural responses, typically catalepsy, hypoactivity or motor dysfunction and hypothermia mediated by actions in the CNS (Thiebot, 1999). This cannabimimetic profile has been established for the naturally occurring THC and

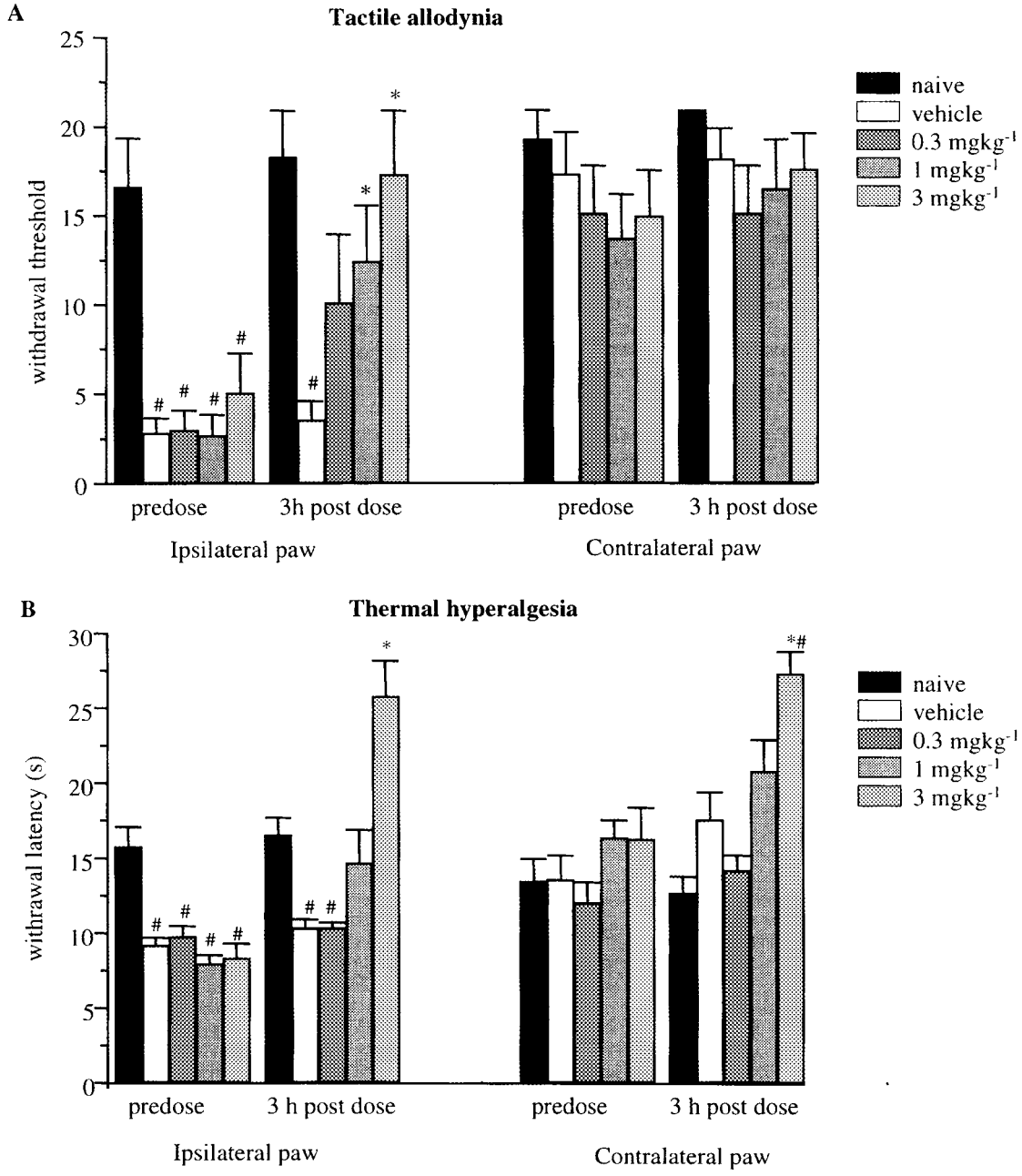


Fig. 7. The effect of WIN55,212-2 on tactile allodynia and thermal hyperalgesia in the partial sciatic ligation model of neuropathic pain. Paw withdrawal thresholds determined using von Frey hairs (A), and withdrawal latencies to a thermal stimulus applied to the plantar surface (B) were measured on the ipsilateral and contralateral paws prior to and 3 h following administration of WIN55,212-2 (0.3–3 mg $kg^{-1}$, s.c.). (A,B) Each column represents the mean ± SEM from six animals per treatment group. *$P < 0.05$ compared to predose reading, and #$P < 0.05$ compared to naive animals by ANOVA followed by Tukey's HSD test.

anandamide, as well as CP-55,940, in the mouse (Compton et al., 1992, 1993), although there is less information on these behavioural effects of cannabinoids in the rat. Part of the aim of the present study was to provide a quantitative analysis of WIN55,212-2, CP-55,940 and HU-210 against neuropathic hyperalgesia, as well as in four assays for central cannabinoid activity in the rat – the tail-flick test for acute antinociception, the rotarod assay for motor dysfunction, the catalepsy ring test and hypothermia. All three compounds were active in this tetrad of tests following systemic administration and their effects were blocked by the $CB_1$ antagonist SR141716A. The rank order of potency observed in these tests was similar to that seen in the neuropathic hyperalgesia assay, with HU-210 being considerably more potent than WIN55,212-2. A comparison of the activities of each compound in all of the behavioural assays shows that each is most potent against neuropathic hyperalgesia after systemic administration, although there is often little separation between their antihyperalgesic activity and activity in one of the tetrad tests. These data indicate a considerable CNS penetration by the cannabinoids. Moreover, alterations in motor activity can possibly affect readings from hyperalgesia assays, which rely on paw withdrawals. These data therefore suggest that potential antihyperalgesic or analgesic compounds with a possible central mechanism of action should be tested also in tests for motor dysfunction to determine a therapeutic window and to ensure specificity of any observed antihyperalgesic activity.

A large body of evidence indicates that the antinociceptive effect of cannabinoids is mediated via a spinal and supraspinal site of action. This is based largely on behavioural and electrophysiological studies following administration of cannabinoids into the spinal cord or discrete brain regions, and is supported by the localization of $CB_1$ receptor mRNA or cannabinoid binding sites in nociceptive regions of the spinal cord and brain (see Richardson, 2000, for review). The mechanisms by which cannabinoids mediate their antihyperalgesic activity are less clear and may involve a combination of central and peripheral activity. In the present study we found that WIN55,212-2, CP-55,940 and HU-210 were all active in the tetrad tests in the same dose range that produced reversal of hyperalgesia, indicating significant CNS penetration. Moreover, WIN55,212-2 reversed neuropathic mechanical hyperalgesia following intrathecal injection via an action at $CB_1$ receptors. This is in agreement with previous studies using inflammatory pain models which have reported that intrathecal delivery of WIN55,212-2 and anandamide inhibits complete Freund's adjuvant-induced allodynia and carrageenan-induced thermal hyperalgesia, respectively (Martin et al., 1999; Richardson et al., 1998b). This inhibitory effect of cannabinoids in the spinal cord could represent an action on primary afferent terminals since anandamide has been shown to inhibit capsaicin-induced sensory neuropeptide release in the isolated spinal cord (Richardson et al., 1998b). An effect on second order neurones is also likely since WIN55,212-2 inhibits the acute response of wide dynamic range neurones in the dorsal horn to peripheral noxious stimuli, as well as the wind-up response to repetitive peripheral stimulation (Strangman and Walker, 1999; Hohmann et al., 1998). It is therefore likely that the antihyperalgesic activity of WIN55,212-2 following systemic administration is at least partly due to an action in the spinal cord.

Recent evidence suggests that cannabinoids may also inhibit inflammatory hyperalgesia via a peripheral site of action. This is based largely on findings that intraplantar injection of anandamide or PEA inhibits formalin-induced nociception or carrageenan-induced thermal hyperalgesia at doses that were ineffective systemically (Calignano et al., 1998; Richardson et al., 1998a). However, there have been no studies examining the site of action of cannabinoids in neuropathic pain. Here we demonstrate a pronounced reversal of neuropathic mechanical hyperalgesia following injection of WIN55,212-2 into the ipsilateral paw. The highest dose used of 100 μg is equivalent to a systemic subcutaneous dose of 0.4 mg $kg^{-1}$ which is close to the $D_{50}$ of 0.5 mg $kg^{-1}$ following systemic subcutaneous administration, and so the intraplantar activity probably represents systemic absorption, especially since a similar degree of reversal was obtained following injection of this dose into the contralateral paw. However, there was a clear separation between the antihyperalgesic effect of 30 μg WIN55,212-2 following ipsilateral or contralateral injection. These data provide some evidence for a peripheral effect of WIN55,212-2, although at higher doses it is clear that there may be systemic absorption and activity at other sites, which may be peripheral or central. As expected, the effect of intraplantar WIN55,212-2 was blocked by systemically administered SR141716A, indicating $CB_1$ receptor involvement. However, intrathecal administration of SR141716A, at a dose that we showed to block the effects of intrathecal WIN55,212-2, did not inhibit the effect of intraplantar WIN55,212-2. This suggests that at least part of the antihyperalgesic effect of WIN55,212-2 observed following intraplantar injection does indeed reflect an action in the periphery rather than absorption into the spinal cord, although a supraspinal effect cannot be ruled out. The obvious target for such a peripheral site of action is primary afferent nerve terminals. In support of this hypothesis, $CB_1$ receptor mRNA has been localized to DRG neurones, and $CB_1$ receptors have been shown to undergo peripheral axonal flow in the sciatic nerve (Hohmann and Herkenham, 1999a,b). Moreover, $CB_1$ receptor activation inhibits sensory neuropeptide release from the skin of rat hindpaws, demonstrating a functional inhibitory activity on peripheral sensory nerves (Richardson et al., 1998a).

Taken together, the findings from the present study show that cannabinoids produce pronounced inhibition of hyperalgesia and allodynia in a model of neuropathic pain in the rat. The evidence suggests that at least part of this effect is mediated via an effect on $CB_1$ receptors located in the periphery, presumably on primary afferent nerve terminals.

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