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The analgesic effects of *R*(+)-WIN 55,212–2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain

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Abstract

The effects of a high affinity cannabinoid receptor agonist were evaluated in rats subjected to chronic constriction injury of the sciatic nerve (CCI) or a sham operation. Intraperitoneal (i.p.) injections of the active, but not the inactive enantiomer, alleviated the pain behavior exhibited by CCI animals in a dose dependent manner. Moreover, at doses ranging from 0.43 to 4.3 mg/kg effects on sensitivity to a heat stimulus were observed neither in the paw contralateral to the sciatic ligation, nor in animals subjected to sham surgery. Animals subjected to CCI and treated with 4.3 mg/kg exhibited hypoalgesia in the paw ipsilateral to the ligated sciatic, i.e. heat hypoalgesia was completely reversed. The hypoalgesia is presumed to be the results of unmasking of a sensory deficit reflecting the known loss of C and A delta with CCI. Although side effects were present in some CCI animals subjected to the high dose (4.3 mg/kg), a moderate dose (2.14 mg/kg) completely alleviated the thermal and mechanical hyperalgesia, and mechanical allodynia without side effects. In addition to identifying a potential drug treatment for painful neuropathy, this study suggests that changes in cannabinoid receptors occurs in nerve injured animals. © 1997 Elsevier Science Ireland Ltd. All rights reserved

Keywords: *R*(+)-WIN 55,212–2; Cannabinoid agonist; Neuropathic pain

The present investigation was initiated to evaluate the potential use of cannabinoids in alleviating the hyperalgesia and allodynia associated with painful peripheral neuropathy. Previous reports indicate that cannabinoids have analgesic [4,5] and anti-inflammatory properties [15], but the studies to date have investigated these properties in anesthetized animals [7] or in normal animals not subjected to chronic neuropathic pain [14]. As the physiological responses of animals subjected to chronic neuropathic pain are likely to be different, further drug evaluation is warranted.

Painful unilateral mononeuropathy was induced in male Sprague–Dawley rats (Taconic Farm, Germantown, NY, USA) weighing 250–350 g as previously described [1]. Briefly, the right sciatic nerve was identified in anesthetized rats and four loose ligatures of 4-0 chromic gut were placed around the nerve trunk. Sham-operated animals underwent similar procedure and four pieces of approximately 5 mm 4-0 chromic gut were placed along the nerve trunk.

Heat-hyperalgesia was assessed in sham-operated ($n = 32$) and chronic constriction injury of the sciatic nerve (CCI) animals ($n = 72$) as described elsewhere [1,6]. The stimulus intensity was adjusted to induce a pre-surgical latency of approximately 10 s. Data are reported as difference scores obtained by subtracting the paw withdrawal latency (PWL) values of the left (unoperated side) from the right hind paw (CCI side) and adjusting to baseline values obtained from individual animals prior to surgery. Thus, negative score values indicate hyperalgesia, positive values indicate hypoalgesia and values close to zero indicate normalization of the response of the hind paw ipsilateral to the ligated nerve to a thermal stimulus. Sham-operated animals showed no change in PWL to a heat stimulus and no significant change from

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pre-surgical baseline PWL values were observed in the paw contralateral to the ligated nerve in CCI animals. Prior to drug administration, on day 9 post-surgery, heat hyperalgesia score was −5.35 ± 0.79.

Nine days following surgery, *R*(+)-WIN 55,212–2 mesylate, a high affinity cannabinoid agonist [1], or *S*(−)-WIN 55,212–3 mesylate, the corresponding inactive enantiomer, were administered intraperitoneally (i.p.), in volumes not exceeding 1.5 ml to CCI or sham-operated animals. Both compounds were dissolved in 45% (w/v) 2 hydroxypropyl-β-cyclodextrin solution. Doses used were: 0.43, 2.14 and 4.3 mg/kg. No changes in PWL to a heat stimulus on the side contralateral to the ligated nerve were observed prior to or following injection of either compound. At the doses tested, no effects were observed on the response to a thermal stimulus in animals subjected to sham surgery. A dose response is demonstrated in Fig. 1; 8–10 CCI animals and four sham-operated animals were used to test the effects of each dose on the response to a thermal stimulus. A dose of 2.14 mg/kg successfully alleviated the hyperalgesia observed ipsilateral to the ligated nerve without affecting the contralateral side. At the higher dose (4.3 mg/kg) hypoalgesia was observed on the side ipsilateral to the ligated nerve, suggesting increased sensitivity to the drug in neuropathic animals on the side ipsilateral to the sciatic ligation. Twenty-four hours following administration, heat-hyperalgesia returned to pre-drug values.

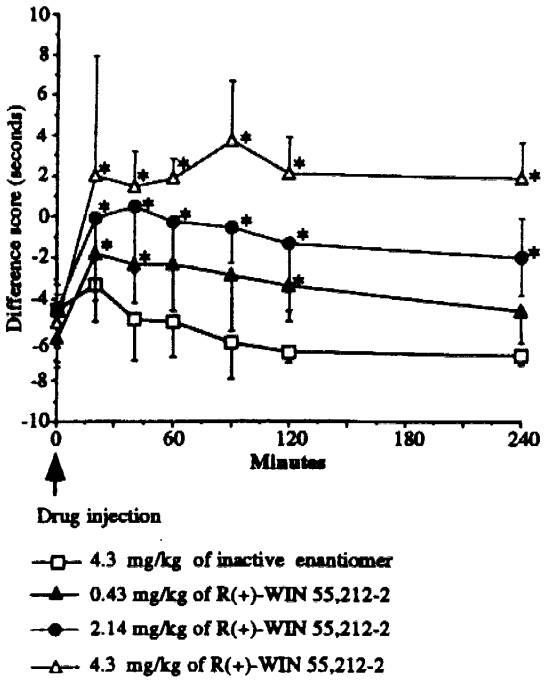

Fig. 1. Responses to a radiant heat stimulus following i.p. injection of *R*(+)-WIN 55,212–2 or its inactive enantiomer. *Significantly different from pre-injection hyperalgesia ($P < 0.01$) as determined by a two-way analysis of variance (ANOVA) and Dunnett's *t*-test.

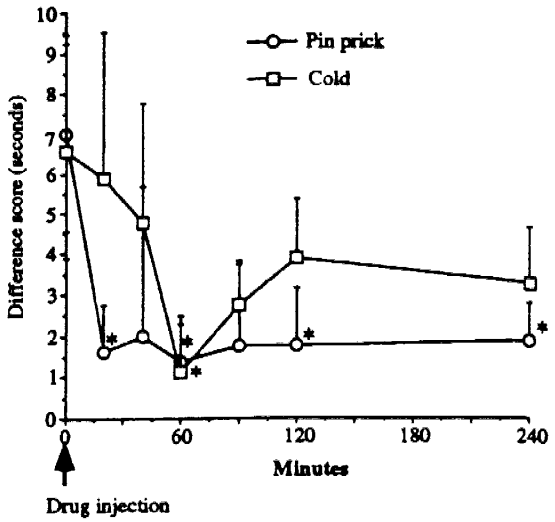

Fig. 2. Responses to cold and pin prick stimuli following i.p. injection of 2.14 mg/kg of *R*(+)-WIN 55,212–2. *Significantly different from pre-drug values at day 8 ($P < 0.01$) as determined by a two-way ANOVA and Dunnett's *t*-test.

Approximately one out of four CCI animals receiving the 4.3 mg/kg WIN 55,212–2 demonstrated side effects. These included loss of motor coordination, increased sensitivity to noise and handling and fear-like responses induced by light touch or handling. No side effects at any dose were observed in animals subjected to sham surgery. The inactive enantiomer showed no analgesic effects or side-effects at any of the doses tested (only the high dose results are presented).

The effects of *R*(+)-WIN 55,212–2 meyslate (2.14 mg/kg) were tested on mechanical hyperalgesia, cold hyperalgesia and mechanical allodynia in eight CCI rats 8 days following surgery. The pin prick test was used to assess mechanical hyperalgesia as described elsewhere [12]. The duration of the withdrawal of the unoperated hind limb was subtracted from that of the operated side to give a difference score. Following the induction of neuropathy, a prolonged paw withdrawal duration was observed in the nerve injured side. This increase was significantly reduced by 2.14 mg/kg *R*(+)-WIN 55,212–2 meyslate, (Fig. 2). Cold hyperalgesia was tested by modifying a procedure described previously [2] and applying 0.2 ml acetone on the plantar surface of the hind paws with a blunted needle. The duration of withdrawal of the unoperated hind limb was subtracted from that of the operated side to give a difference score. An increase in the duration of withdrawal was observed following CCI surgery. This increase was also significantly reduced to pre-surgical levels by the drug (Fig. 2). Mechanical allodynia was tested with von Frey hairs as described [8]. The log value of the bending force (in mg) required to elicit a response from the unoperated leg was subtracted from that of the operated one, giving a log ratio comparing injured to non-injured hind limb

sponse to a mechanical stimulus. As with the thermal nd mechanical hyperalgesia, no changes in the side contralateral to the ligated or sham operated nerve were bserved prior to or following drug treatments. As with ne thermal and mechanical hyperalgesia, 2.14 mg/kg of R(+)-WIN 55,212–2 meyslate significantly alleviated the nechanical allodynia (Fig. 3) and as with the thermal yperalgesia, 24 h following administration, mechanical yperalgesia and allodynia and response to a cold stimulus eturned to pre-drug values.

To further characterize the specific receptor mediating he anti hyperalgesic effects of WIN 55,212–2, a CB1 receptor antagonist, SR141716A [10] was injected (i.p.) to sham-operated as well as to animals subjected to CCI. The CB1 antagonist was injected alone, (0.5 mg/kg) or together with 2.14 mg/kg WIN 55,212–2 (see Figs. 4 and 5). When injected alone, the cannabinoid antagonist exacerbated the hyperalgesia and mechanical allodynia by preferentially lowering the injured hind paw withdrawal latency to heat stimulus and lowering its the threshold response to von Frey filaments. At a dose of 0.5 mg/kg, SR141716A had no effects on the non-injured hind paw of CCI animals or on either hind paws of sham operated animals. That dose also completely reversed the effects of WIN 55,212–2 on mechanical allodynia (Fig. 5) and partially reversed the effects of WIN 55,212–2 on thermal hyperalgesia (Fig. 4). At a higher dose of, 5 mg/kg, SR141716A exacerbated both the mechanical allodynia and the thermal hyperalgesia in the face of 2.14 mg/kg of WIN 55,212–2.

The findings presented in this report demonstrate an increased sensitivity of neuropathic animals to a cannabinoid agonist and antagonist. Whereas sham operated animals showed no changes in PWL to a thermal stimulus following i.p. administration of *R*(+)-WIN 55,212–2 meyslate, thermal hyperalgesia was significantly alleviated in CCI animals and hypoalgesia in the hind limb subjected to nerve injury was induced when a dose of 4.3 mg/kg was used. Previous reports have demonstrated that *N*-methyl-D-aspartate (NMDA) receptor antagonists relieve the hyperalgesia and allodynia associated with unilateral mononeuropathy [8,11,13]. HU-211, a cannabinoid agonist has been shown to attenuate NMDA receptor-mediated neurotoxicity [9]. Our findings, on the other hand, seem to be mediated via a CB1 cannabinoid receptor, as the anti hyperalgesic effects of WIN 55,212–2 were completely reversed by a specific CB1 cannabinoid antagonist. It is still possible that the anti hyperalgesic effects of the cannabinoid agonist used in our experiment are mediated by a pre-synaptic inhibition of glutamate release, thus preventing glutamate actions on a post synaptic NMDA receptor. Cold hyperalgesia was the measure least sensitive to the analgesic effects of the cannabinoid (Fig. 2). As cannabinoids produce hyperthermia [3], an increased body temperature and therefore an increased sensitivity to a cold stimulus might account for this result. A more perplexing observation emerging from our experiments are the behavioral side effects sometimes occurring,

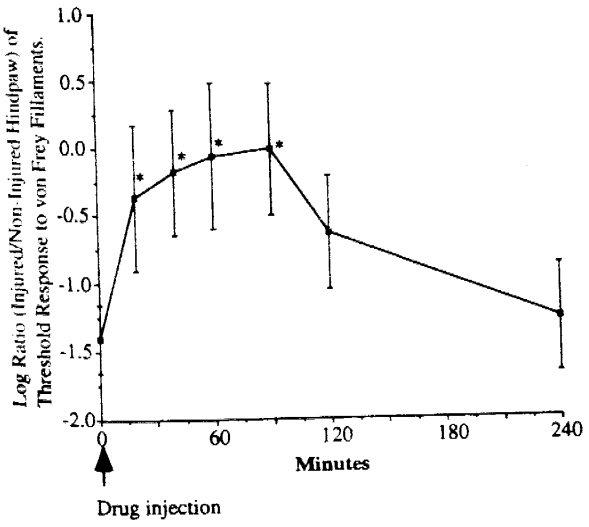

Fig. 3. Responses to graded von Frey filaments following i.p. injection of 2.14 mg/kg of *R*(+)-WIN 55,212–2. *Significantly different ($P < 0.01$) from pre-drug values at day 8 as determined by a two-tailed Wilcoxon rank test.

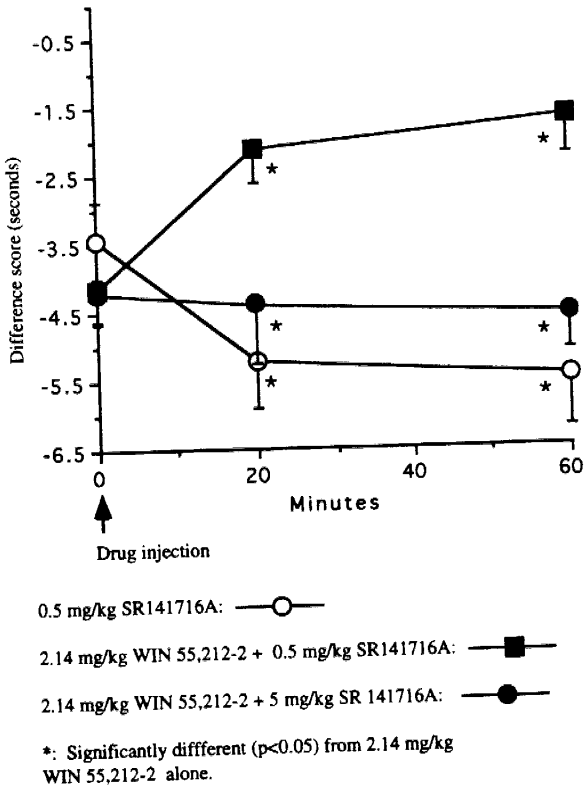

Fig. 4. Responses to a radiant heat stimulus following i.p. injection of drug combinations illustrated in the figure. *Significantly different ($P < 0.05$) from 2.14 mg/kg WIN 55.212–2 alone.

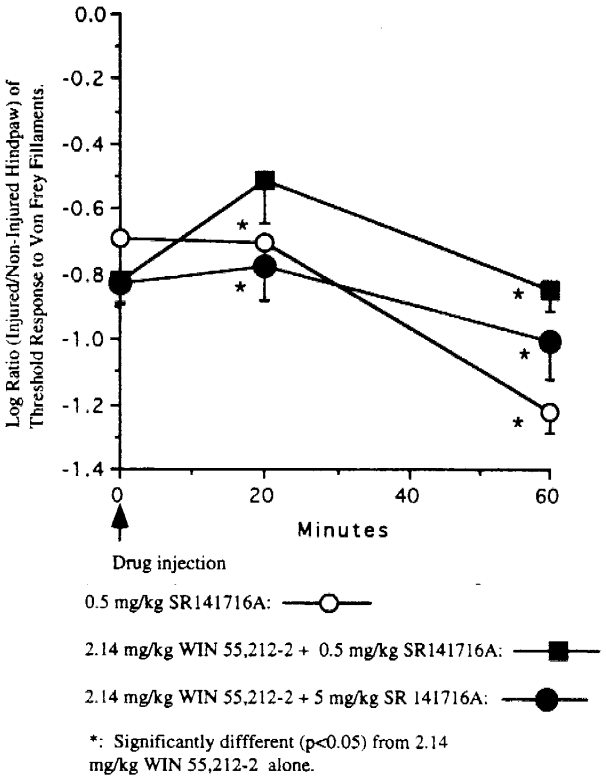

Fig. 5. Responses to von Frey filaments following i.p. injection of drug combinations illustrated in the figure. *Significantly different ($P < 0.05$) from 2.14 mg/kg WIN 55,212-2 alone.

only in CCI animals, when the high dose of the cannabinoid agonist (4.3 mg/kg) was administered. All sham-operated animals seemed unaffected by the drug at doses up to 20 mg/kg. Whether this finding indicates a central up-regulation of the cannabinoid receptor in CCI rats also remains to be determined.

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