The effect of WIN 55,212-2, a cannabinoid agonist, on tactile allodynia in diabetic rats

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Abstract

The antinociceptive action of cannabinoids in acute and inflammatory pain states have been well-documented. There is also accumulating evidence suggesting that cannabinoids are effective analgesics in chronic pain conditions. WIN 55,212-2, a mixed CB1 and CB2 cannabinoid receptor agonist, has been shown to be effective against hyperalgesia and allodynia in painful peripheral mononeuropathy. Recently, in addition to their spinal and supraspinal antinociceptive action, cannabinoids have also reported to exert local analgesic effects. The aim of this study is to observe the effect of a high affinity cannabinoid, WIN 55,212-2, on tactile allodynia and thermal hyperalgesia in diabetic rats. Diabetes was produced with the injection of a single dose of streptozocin (50 mg/kg, i.p.) and this procedure resulted in neuropathic pain behaviors in the hindlimbs. Mechanical allodynia was detected by application of von Frey filaments to the plantar surface of the foot, and thermal hyperalgesia was studied using the Hargreaves’ method; however, thermal hyperalgesia did not develop in diabetic rats. With its higher doses, both systemic (3 and 10 mg/kg, i.p.) and peripheral (30 µg, i.p.l.) injections of WIN 55,212-2 reduced mechanical allodynia. These results suggest that WIN 55,212-2 has an antiallodynic effect in streptozocin-induced diabetic rats and may be a promising approach in the treatment of diabetic neuropathy.

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For centuries cannabis has been used for the management of pain; however, we began to understand how cannabinoids affect neural function, only after the recent discovery of cannabinoid receptors and their putative endogenous ligands [19,24]. It is suggested that cannabinoids within the central nervous system may have a neurotransmitter or neuromodulator function, and there is now considerable evidence supporting a role for cannabinoids in nociception, particularly in chronic pain conditions [14,19]. However, the mechanisms by which the cannabinoids produce analgesia are as yet unclear. Two subtypes of cannabinoid receptors have been identified and cloned: CB1 and CB2. CB2 receptors have been reported to modulate inflammation. On the other hand, CB1 receptors have been demonstrated both in the central nervous system, including areas of brain and spinal cord associated with nociception, and in certain peripheral tissues [12,19,24].

The naturally occurring Δ9-tetrahydrocannabinol, synthetic cannabinoids such as WIN 55,212-2, and putative endocannabinoids such as anandamide and 2-arachinoylglycerol have been shown to exert analgesic effects in different rat models of pain [11,14,19,24].

Like peripheral nerve injury models, diabetes may also lead to neuropathic pain, which is characterized by spontaneous pain, hyperalgesia (augmented pain response to normally painful stimuli), and allodynia (nociceptive responses to normally innocuous stimuli) [5,15]. These behavioral signs, which are analogous to clinical conditions of neuropathic pain, have been shown to develop in a widely employed animal model of neuropathy: streptozocin (STZ)-induced diabetic neuropathy [3,5]. Recently, the cannabinoid receptor agonist, WIN 55,212-2 has also shown to be effective against hyperalgesia and allodynia in painful unilateral mononeuropathy [2,14]. Moreover, it is reported that contralateral thalamic cannabinoid CB1 receptors are upregulated after a rat model of chronic neuropathic pain, and it is hypothesized...
that cannabinoid CB1 receptor upregulation contributes to the increased analgesic efficacy of cannabinoids in chronic pain conditions [16]. However, the effects of cannabinoids on behavioral signs of diabetic neuropathy have been studied only recently [8].

The aim of this study is, therefore, to observe the effect of a high affinity cannabinoid agonist, WIN 55,212-2, on tactile allodynia and thermal hyperalgesia in diabetic rats. We planned to observe this effect of WIN 55,212-2, both peripherally and systemically. This attempt may provide clinicians the use of local cannabinoids as a new treatment option in diabetic neuropathy.

This study was conducted according to the guidelines of the Ethical Committee of the International Association for the Study of Pain, and had been approved by the “Animal Care Ethics Committee” of our faculty. Male Wistar rats (DE-TAM, Istanbul, Turkey), weighing 300–350 g at the time of injection, were used for the experiments. STZ-induced neuropathic pain model, previously described by Courtie et al. [5], was used. Tactile allodynia is known to develop in this animal model of diabetes [3,5]. One week after STZ injection (50 mg/kg, i.p.), diabetes was confirmed by determining blood glucose levels in blood samples taken from tail veins, using Glucometer Elite (Bayer) test strips. Rats with blood glucose levels ≥12 mM were considered diabetic and included into the study.

Tactile allodynia thresholds were assessed, as described previously [4,22]. Briefly, the rats were placed under a glass cover on a metal mesh floor 15 min before the tests and adapted to testing environment. Eight von Frey filaments, with approximately equal logarithmic incremental bending forces, were chosen (von Frey numbers: 3.22, 3.61, 4.08, 4.31, 4.56, 4.93, 5.18, 5.46; equivalent to 0.16, 0.4, 1, 2, 4, 8, 15 and 26 g). Testing was initiated with the 2.0 g hair, and each hair was pressed perpendicularly against the plantar surface of the hindpaw until slight buckling was observed. Lifting of the paw was recorded as a positive response, and 50% withdrawal thresholds were determined using the up-down method of Dixon [6]. If the rat responded, the next weaker hair was applied. In case of a negative response, the next stronger hair was applied and the test was continued until four measurements after the first change in response had been obtained. The pattern of positive and negative responses was converted into a 50% threshold value using the formula given by Dixon [6]: 50% threshold = \( \frac{X_{\bar{f}} - X_{t}}{x} \), where \( X_{\bar{f}} \) = value of the final von Frey hair used (in log units), \( X_{t} \) = tabular value for the pattern of positive/negative responses, and \( \bar{f} \) = mean difference in stimuli in log units (0.3).

Thermal nociceptive threshold to radiant heat was quantified using the method of Hargreaves et al. [13]. Briefly, rats were placed in a plexiglass box on top of the temperature maintained (30 ± 0.1 °C) glass surface of the stimulator (Maycom, Turkey). A beam of radiant heat was applied through the glass to the plantar surface of the hind paw. The paw withdrawal latency, defined as the time from onset of the radiant heat to the withdrawal of the paw, was detected with a photocell and a timer. The radiant heat source was adjusted to result in pre-injection latencies of 12–13 s. A maximal cut-off of 20 s was used to prevent tissue damage.

Tests took place two to four weeks after STZ injection. Tactile allodynia thresholds were assessed immediately before, and at 0.5, 1, and 2 h after i.p. (1–10 mg/kg) and i.p. (3–30 μg) injections of WIN 55,212-2. Control rats received a similar administration of 50% DMSO in saline. In case of peripheral injections, drugs were administered s.c. to the dorsal surface of the right hind paw in a volume of 50 μL, and the von Frey filaments were applied to the ventral surface of the same paw [23]. In order to determine whether the drugs were acting peripherally, the same doses were also administered to the left hind paw, and the same procedure was applied to the right hind paw. The same person performed each assessment in order to minimize the differences in technique.

WIN 55,212-2, DMSO and STZ were purchased from Sigma Chemical Co. STZ was dissolved in isotonic NaCl and WIN 55,212-2 was dissolved in 50% DMSO in saline. One-way analysis of variance (ANOVA) followed by Newman–Keuls test was used for analyzing the data from tactile allodynia. Values of \( P < 0.05 \) were considered to be significant. All data were expressed as mean ± S.E.M.

As described before [3,5], STZ-injected rats developed pain-related behavior, marked as tactile allodynia. The 50% paw withdrawal thresholds prior to STZ injection was 13.55 ± 1.5. Three weeks after STZ administration, significant reductions in paw withdrawal thresholds to von Frey filaments were observed (2.6 ± 0.6, \( P < 0.05 \), Fig. 1 A and B). On the other hand, there were no changes in thermal nociceptive thresholds during a period of one month after STZ injection. Pre-injection latency to radiant heat was 12.6 ± 1.6, and did not change during the experiments. The animals did not lose weight throughout the experiments, but one rat died during this study.

At higher doses, both systemic (3 and 10 mg/kg, i.p.) and peripheral (30 μg, i.p.) administrations of WIN 55,212-2 produced increases in paw withdrawal thresholds, compared...
than acute pain [12, 19, 24]. Accordingly, WIN 55,212-2, a high affinity cannabinoid agonist, has been shown to exert antihyperalgesic and antiallodynic effect in nerve injury-induced neuropathic pain [2, 14]. In contrast, much less is known of the effects of cannabinoids in diabetes-induced neuropathic pain [8]. We studied the effect of WIN 55,212-2 only on tactile allodynia, because, similar to previous reports [10, 18], thermal hyperalgesia did not develop in our diabetic rats.

Cannabinoids modulate pain by acting at spinal and supraspinal sites [20]. Current evidence also indicates an action of cannabinoids in the periphery [7, 20]. Topical cannabinoid antinociception and its synergy with spinal sites and topical morphine treatment have been indicated [7, 27]. Topical cannabinoid treatment offers some advantages, since their side effects limit the systemic use of these drugs as effective analgesics.

Although similar behavioral symptoms, such as allodynia and hyperalgesia, are observed both in nerve injury- and STZ-induced neuropathic pain, it is generally accepted that tactile allodynia of diabetic rats may not share the same etiology with tactile alldyenia of nerve-injured neuropathic rats [3, 9]. Inconsistent results are obtained with the use of different drugs in these two pain states [3, 9]; however, our results from STZ-induced neuropathic pain were similar to those obtained from nerve injury-induced neuropathic pain. We observed that systemic injection of WIN 55,212-2, with its higher doses tested, reduces tactile alldyenia in STZ-induced diabetic rats. Peripheral action of WIN 55,212-2 in diabetic neuropathy must arouse interest, since this data suggest a novel treatment strategy for diabetes-induced neuropathic pain.

Cannabinoids are known to produce a variety of centrally mediated effects such as deficit in motor performance [10, 14]; however, these results were obtained from nerve-injured, not diabetic, rats. Although, Herzberg et al. [14] showed that WIN 55,212-2 demonstrated side effects such as loss of motor coordination, increased sensitivity to noise and handling and fear-like responses induced by light touch or handling, they also showed that no side effects at any dose of WIN 55,212-2 were observed in animals subjected to sham surgery. It can be speculated from these results that motor side effects of WIN 55,212-2 is significant, especially in nerve-injured neuropathic animals. Although we did not evaluate the rotarod performance or the other tetrad models, we did not observe any motor incoordination during our experiments, supporting the difference in the etiology of diabetic neuropathy and nerve injury-induced neuropathy.

In conclusion, our results demonstrate that both systemically and peripherally administered WIN 55,212-2 exerts anti-allodynic effect in diabetic rats with its higher doses. While it should be emphasized that further experiments must be carried out to delineate the mechanism of the anti-allodynic property of WIN 55,212-2, the results of this study indicate that local use of cannabinoids should be kept in mind as an alternative therapy for symptomatic relief in diabetic neuropathy.

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References


