# Characterization of cannabinoid-induced relief of neuropathic pain in rat models of type 1 and type 2 diabetes

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ABSTRACT

Diabetic neuropathy is a frequent complication of diabetes mellitus with a tremendous impact on patients' quality of life, and it remains poorly treated. Cannabinoids relieve the signs of diabetic neuropathy in different experimental models, including streptozotocin- (STZ-) induced type 1 diabetic rodents, and they may also relieve neuropathic signs in type 2 diabetic animals. This study compares the effect of the non-selective cannabinoid agonist WIN 55,212-2 (WIN) in Zucker Diabetic Fatty (ZDF) rats (type 2 diabetes) and in STZ-injected Wistar rats (type 1 diabetes).

WIN (or its vehicle) was either systemically administered at a non-psychoactive dose or locally injected. Selective CB1 and CB2 cannabinoid antagonists were used to characterize WIN antineuropathic effects.

Both type 1 and type 2 diabetic rats showed mechanical allodynia but not thermal hyperalgesia. WIN alleviated mechanical allodynia in both models of diabetes. In STZ-treated rats, both cannabinoid receptors were involved, whereas in ZDF rats, WIN effects seemed to mainly involve the activation of CB1 receptors. Higher doses of WIN were needed to significantly relieve mechanical allodynia upon intraplantar administration in ZDF vs. STZ-injected rats.

Cannabinoids, acting on systemic and/or peripheral receptors, may serve as a new therapeutic alternative for symptom management in painful neuropathy associated with both type 1 and type 2 diabetes. Additionally, our results highlight the need for appropriate selection of diabetic experimental models because the results from studies in STZ-induced diabetic rodents might not be applicable in all diabetic situations.



## 1. Introduction

Diabetic neuropathies are among the most frequent complications of diabetes mellitus (Aring et al., 2005) and have a tremendous impact on patients' quality of life. Patients with diabetic sensory neuropathy experience a variety of aberrant sensations including spontaneous pain, hyperalgesia (increased pain from a stimulus that normally provokes pain) and allodynia (pain due to a stimulus that does not normally provoke pain, like the touch of clothes or bed sheets) (IASP taxonomy; Vinik et al., 1995).

The prevalence of neuropathy in diabetic patients may vary between studies (50–60%, Talbot et al., 2010; 16%, Bril et al., 2011). It is frequently unreported (12.5%) and more frequently untreated (39%) (Bril et al., 2011). Furthermore, although effective treatments for diabetic neuropathy are available (see Bril et al., 2011; Smith and Argoff, 2011), many have side effects that limit their usefulness. Therefore, alternative therapies are still needed.

The cannabinoid system is one of the endogenous systems that modulate pain perception. However, the clinical use of cannabinoids is limited by their psychoactive properties, mediated by cannabinoid receptors expressed in the central nervous system (CNS). One way to dissociate cannabinoid analgesia from the psychoactive effects is to target cannabinoid receptors type 1 (CB1R) located in peripheral nerve fibers (Karst and Wippermann, 2009). Topical application of cannabinoids has reduced pain in a human experimental model (Rukwied et al., 2003). In animal models, local administration of CB1R agonists has been reported to produce anti-nociceptive effects in both inflammatory and neuropathic conditions (Fox et al., 2001; Nackley et al., 2003; Richardson et al., 1998).

Most preclinical studies for diabetic neuropathy have been carried out in streptozotocin- (STZ-, a pancreatic beta-cell cytotoxin) induced diabetic rodents. These rodents develop a syndrome that resembles type 1 diabetes. Cannabinoids have been tested in this model of diabetes (Doğrul et al., 2004; Ulugol et al., 2004; Bujalska, 2008). In STZ-induced diabetic rats, the non-selective cannabinoid agonist WIN 55,212-2 (WIN) reduced mechanical allodynia and hyperalgesia (Ulugol et al., 2004; Bujalska, 2008).

Several preclinical studies have examined diabetic neuropathy in type 2 diabetic animals (Otto et al., 2011; Oltman et al., 2008; Romanovsky et al., 2008). These models are important in the studies of diabetic complications. In adult humans, type 2 diabetes accounts for about 90% to 95% of all diagnosed cases of diabetes (National

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Diabetes Statistics, 2011). Animal studies of type 2 diabetes can provide useful information on the progression of nerve dysfunction in diabetic patients (Shaikh and Somani, 2010). To our knowledge, no study has been carried out to test the antinociceptive effect of cannabinoids in an animal model of type 2 diabetes, such as the Zucker Diabetic Fatty (ZDF) rat.

Therefore, the aim of this work was to determine whether the non-selective cannabinoid agonist WIN 55,212-2 (WIN) is able to alleviate the signs of sensory neuropathy associated with long-term diabetes in ZDF rats. Additionally, the involvement of CB1 and CB2 receptors was investigated. Furthermore, a parallel study was carried out in STZ-injected rats.

## 2. Methods

The experiments in the present study were designed to minimize the number of animals used and their suffering, and throughout the experimental procedure, the international ethic standards for pain-inducing experiments in laboratory animals (Zimmermann, 1983) and the European Communities Council Directive (No. 86/609, Nov 24, 1986) were followed. All animal procedures were reviewed and approved by the Animal Care and Ethical Committee of Universidad Rey Juan Carlos.

### 2.1. Models of diabetes and diabetic neuropathy

Male Wistar rats (240–300 g) obtained from Harlan Laboratories (Barcelona, Spain) were used to induce type 1 diabetes. For type 2 diabetes, obese diabetic male ZDF/crl-lepr/fa and control lean male *fa*/+ rats were purchased from Charles River Laboratories (Research Models, Barcelona, Spain) and shipped to the Universidad Rey Juan Carlos at 6 weeks of age.

Upon arrival at our laboratory, animals were stored (2–4/cage) in standard transparent cages (60 cm × 40 cm × 20 cm) furnished with wood shaving bedding, which was changed three times a week. Cages were placed adjacent to each other, in a climate-controlled environment (temperature = 20 °C; humidity = 60%) with a 12 h light/12 h dark cycle (lights on between 08:00 and 20:00 h). Animals had free access to standard laboratory rat chow (Harlan-Iberica: Wistar rats) or Purina rat chow 5008 (Charles River: ZDF rats and their controls) and to sterile tap water.

One week after arrival to the laboratory, diabetes was induced in Wistar rats by intraperitoneal administration of STZ at a dose of 60 mg/kg body weight (Suanarunsawat et al., 1999). STZ was dissolved in citrate buffer at pH 4.5 and administered in one dose on the first day of the study. Following the injection, food and water were available *ad libitum* during the remaining 28 days of the experiment. Control rats received an equal volume of buffer (1 ml/100 g of body weight).

Non-fasting blood glucose levels were measured using a glucose strip tester (Glucocard™ sensor, Arkray, Inc. Kyoto, Japan) applied to blood samples drawn from the tail vein. In Wistar rats, this was performed on day 21 after STZ or buffer injection. At this time point, hyperglycemia was not developed in only 4 out of 55 STZ-injected rats and these rats were discarded. Therefore, 51 STZ- and 16 buffer-injected rats were used in the behavioral tests (see below).

In ZDF rats (n = 20) and their lean controls (n = 16), glucose levels were recorded at three different time-points in order to check for diabetes development (Clark et al., 1983; González et al., 2011; Peterson et al., 1990): 7–8 (pre-diabetes: hyperglycemia and insulin resistance begin to develop), 12–13 (short-term diabetes) and 16–17 weeks of age (long-term diabetes: at 15–16 weeks of age, glucose levels typically reach a plateau of more than 300 mg/dl in ZDF rats). No rat was discarded in this case. Glucose levels were determined on the day after the behavioral tests to avoid stress.

The occurrence of neuropathic signs (mechanical allodynia, heat hyperalgesia) was monitored on days 21 and 28 post-injection (type 1 diabetes; Bujalska, 2008) or at 7–8, 12–13 and 16–17 weeks of age (type 2 diabetes).

Mechanical sensitivity was assessed using an electronic Von Frey apparatus (EVF3, Bioseb, BP89, Chaville Cedex, France). Rats were placed individually on an elevated iron mesh in a clear plastic cage and were allowed to adapt to the testing environment for at least 10 min. Using a filament connected to the Von Frey apparatus, a mechanical stimulus of increasing intensity was applied to the plantar aspect of each hindpaw, through the mesh floor. The apparatus automatically records the intensity of the stimulus that provokes the sudden withdrawal of the paw, and has an upper cut-off limit of 50 g. The test was performed four times with an *interstimulus* interval of approximately 1 min. The mean of the four trials was used for data analysis. Mechanical allodynia was defined as a significant decrease in the withdrawal threshold evoked by mechanical stimuli.

Heat-antinociception was tested using a 37370 plantar test apparatus (Ugo Basile, Comerio VA, Italy). The withdrawal latency from a focused beam of radiant heat applied to the mid plantar surface of the hindpaws was recorded. The intensity of light was adjusted at the start of the experiment such that the control average baseline latencies were about 8 s and a cut-off latency of 25 s was imposed. The withdrawal latency of each paw was measured during three trials at 5 min intervals, and the mean of the three readings was used for data analysis.

Animals were habituated to the corresponding test environments, two days before the experiment, by leaving them inside the recording device for ten minutes.

### 2.2. Evaluation of the cannabinoid antinociceptive effect in diabetic rats

On day 28 after STZ injection (type 1 diabetes), or at 16–17 weeks of age (type 2 diabetes), three sets of experiments were carried out in the diabetic rats to test and characterize the antinociceptive effect of WIN. First, in some rats a single dose of vehicle (1 ml/kg; n = 7) or WIN (1 mg/kg; n = 8) was intraperitoneally administered. Second, some diabetic rats received an intraperitoneal (i.p.) injection of the CB1 (AM251) or the CB2 (SR144528) antagonists (1 mg/kg; n = 8 each group) 20 min prior to WIN/vehicle i.p. injection. The effect of the drugs intraperitoneally administered was analyzed in both paws. Finally, some diabetic rats received an intraplantar (i.pl.) administration of a single dose of vehicle (25 μl) or WIN (50 or 100 μg) alone or 20 min after the intraperitoneal injection of either antagonist (n = 6 each group); i.pl. administration took place in the right hindpaw and the left paw served as control. The nociceptive behaviors were tested 20 min after WIN i.pl. administrations by an observer unaware of the treatments, as described in the previous section.

### 2.3. Psychoactive effects of WIN 55,212-2 (cannabinoid tetrad)

The classical cannabinoid tetrad test (Compton et al., 1993) was applied to monitor the psychoactive effects of WIN (1 mg/kg, i.p.). In previous studies this dose was effective to relieve neuropathic pain (Pascual et al., 2005; Vera et al., 2007; Bujalska, 2008), and practically devoid of psychoactivity in control Wistar rats (Vera et al., 2007). Thus, the effect of this dose was also tested in the other group of control animals (lean rats, n = 4) and both types of diabetic rats (n = 6, each group). The test values were recorded by an observer unaware of the treatments, as previously reported (Abalo et al., 2009, 2010).

Heat-antinociception of the hindpaws was tested 20 min after drug administration, as described above (plantar test).

To measure catalepsy, the rats were hung by their front paws from a rubber-coated metal ring (12 cm diameter) fixed horizontally at a

height that allowed their hindpaws to just touch the bench. The time taken for the rat to move off the ring was measured with a cut-off limit of 30 s. Latencies were measured 25 min after drug or vehicle administration.

Core temperature was recorded 30 min after drug administration using a P6 thermometer and a lubricated rectal probe (Cibertec S.A., Madrid, Spain) inserted into the rectum to a constant depth of 5 cm.

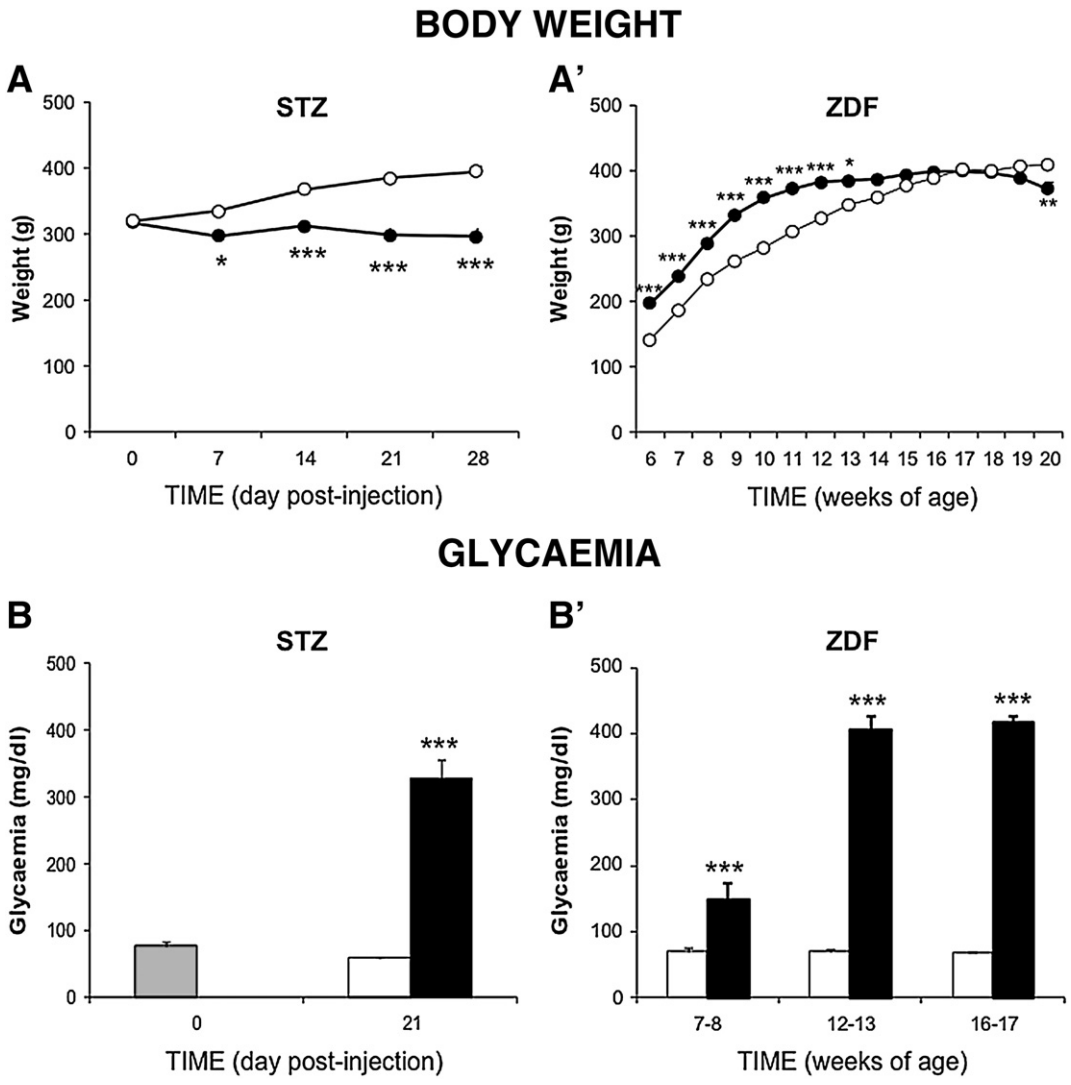
Spontaneous locomotor activity was evaluated using individual photocell activity chambers (Cibertec S.A., Madrid, Spain). Rats were placed in the recording chambers (55×40 cm, spacing between beams 3 cm) 40 min after drug administration, and the number of interruptions of photocell beams was recorded over a 30-min period. To make sure that the effects of WIN on neuropathic signs were not due to a time-dependent alteration in spontaneous locomotor activity, this test was also performed in non-treated Wistar rats 20 min after administration of WIN, the same time point at which mechanical and thermal sensitivity were recorded. In this case, both the total number of activity counts throughout the 30 min of test duration and the number of activity counts recorded for the first 5 min were recorded.

### 2.4. Compounds and drugs

WIN 55,212-2 was obtained from Tocris Cookson (Bristol, U.K.) and AM251 was purchased from Ascent Scientific Ltd. (North Somerset, BS24 9 ES, UK). SR144528 was kindly gifted by Sanofi Aventis Recherche & Developpement (Montpellier, France). All these drugs were dissolved in Tocrisolve (a commercially available water soluble emulsion composed of a 1:4 ratio of soya oil/water that is emulsified with the block co-polymer Pluronic F68; Tocris, Cookson, Bristol, UK), which served as vehicle. Streptozotocin was purchased from Sigma (Sigma Aldrich Química, S.A., Madrid, Spain) and dissolved in citrate buffer (Panreac Química, S.A., Barcelona, Spain) at pH 4.5.

### 2.5. Statistical analysis

Data are presented as the mean values ± the standard error of the mean (SEM). Differences between groups were analyzed using one-way or two-way ANOVA followed by the *post-hoc* Bonferroni multiple comparison test. Values of $p<0.05$ were regarded as being significantly different.



## 3. Results

### 3.1. Development of diabetes and diabetic neuropathy

At the beginning of the experiment, Wistar rats had a body weight of approximately 320 g. Twenty eight days after STZ administration, the body weight of vehicle-injected rats was almost 400 g, whereas that of the diabetic rats did not significantly change (Fig. 1A). Initially, ZDF rats had a body weight of almost 200 g, which increased for the first few weeks after arrival to the laboratory, and reached a plateau of approximately 400 g at 12–13 weeks of age. In contrast, their lean controls originally had a body weight of approximately 140 g that increased throughout the whole experiment and showed values similar to or even higher than those of ZDF rats from 15 to 16 weeks of age onwards (Fig. 1A′).

Control (vehicle-injected or lean) animals had a random (non-fasting) blood glucose concentration of less than 100 mg/dl throughout the whole experiment. In contrast, random glycemia was higher than 300 mg/dl 21 days after STZ administration (Fig. 1B). All ZDF rats were already significantly hyperglycemic at the first time point evaluated (7–8 weeks), but glycemia continued to increase, reaching values of 400 mg/dl at 12–13 weeks of age and onwards (Fig. 1B′).

As shown in Fig. 2A and A′, mechanical sensitivity remained constant for control (vehicle-treated and lean) rats throughout time. In STZ-injected animals, mechanical allodynia was evident at both 21 and 28 days after induction of diabetes (Fig. 2A). In ZDF rats (Fig. 2A′), the withdrawal threshold for mechanical stimulation was already significantly lower from the first time-point evaluated (7–8 weeks of age), although the difference increased at longer time-points (12–13 and 16–17 weeks of age).

No sign of thermal hyperalgesia was detected in STZ-injected (Fig. 2B) or ZDF (Fig. 2B′) rats compared to their respective controls. Moreover, ZDF rats showed a slight but significant hypoalgesia at 16–17 weeks of age compared to their lean controls (Fig. 2B′).

### 3.2. Effect of acute intraperitoneal administration of WIN 55,212-2 and cannabinoid antagonists on diabetic neuropathy

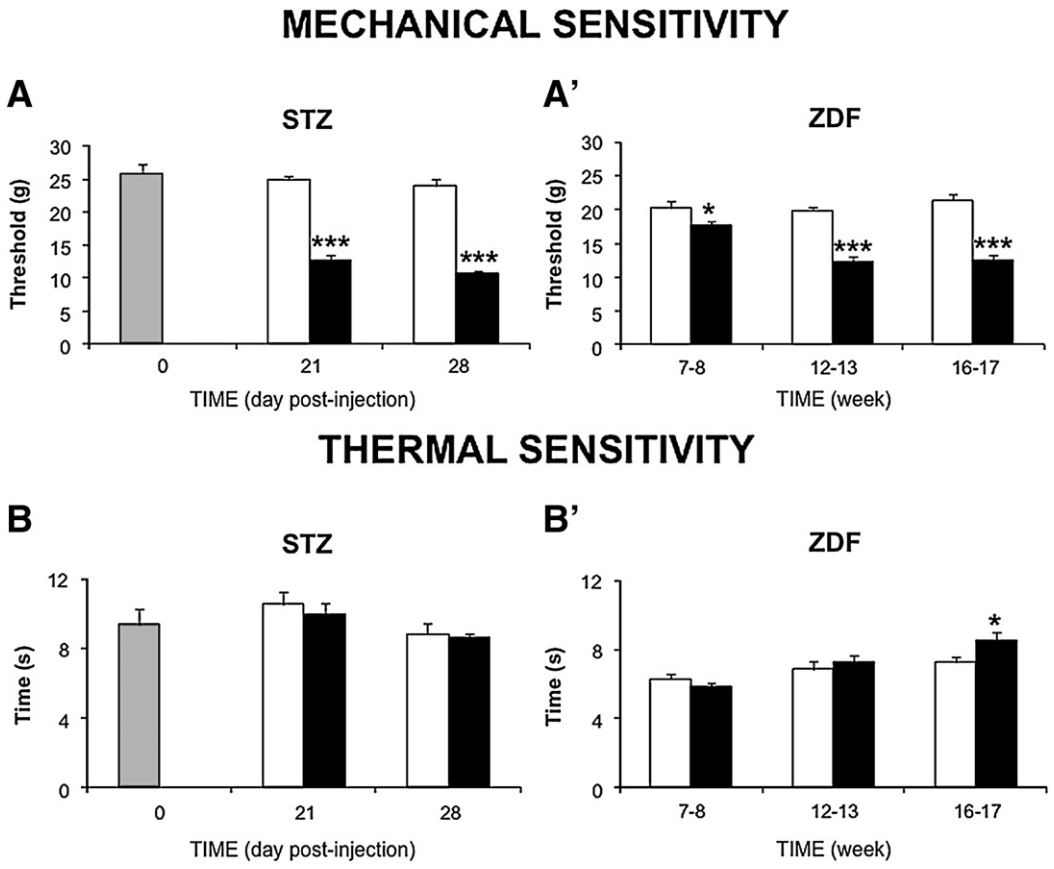
The effect of WIN was evaluated in diabetic (STZ-injected and ZDF) rats showing neuropathic signs.

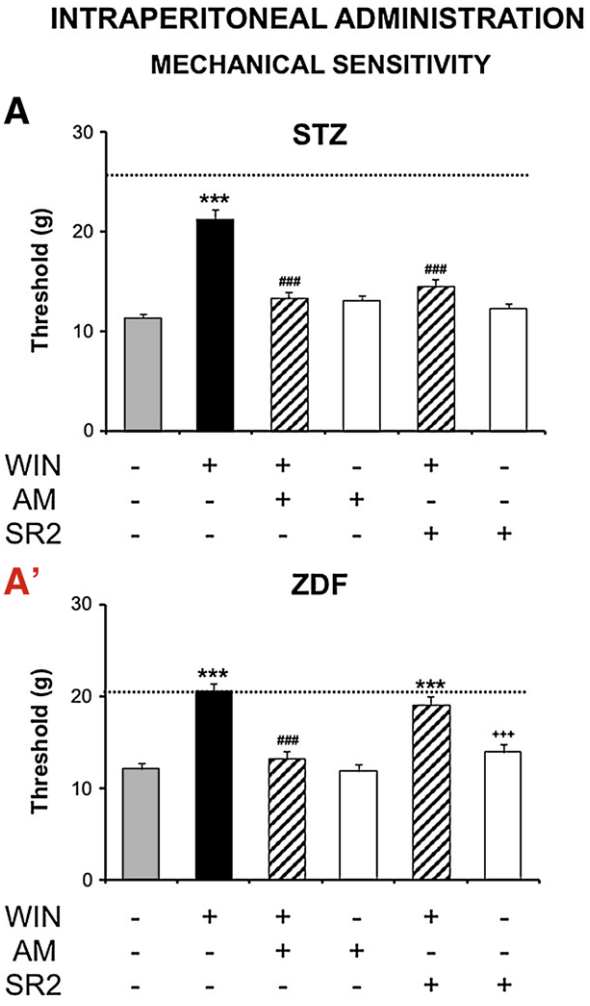
In both STZ-injected and ZDF rats (Fig. 3), acute intraperitoneal administration of WIN reversed mechanical sensitivity to the values typical of their respective control, non-diabetic rats. However, whereas the WIN effect was sensitive to both CB1 and CB2 antagonists in STZ-injected rats (Fig. 3A), the CB2 antagonist was unable to block it in ZDF animals (Fig. 3A′). When given alone, no antagonist significantly altered the withdrawal threshold for mechanical stimulation compared to that in vehicle-treated diabetic rats.

In the plantar test, none of the treatments produced withdrawal times significantly different from the WIN vehicle in the STZ model or ZDF rats (data not shown).

### 3.3. Effect of WIN 55,212-2 locally administered

Upon intraplantar administration, WIN increased the withdrawal threshold to mechanical stimulation in the ipsilateral paw of STZ-injected (Fig. 4A) or ZDF rats (Fig. 4A′). In STZ-injected animals, the lowest dose tested (50 μg) was already effective and no further increase in threshold was obtained with the highest WIN dose






(100 μg). In the case of ZDF rats, the antiallodynic effect was significant only at the highest dose tested (100 μg).

As for intraperitoneal administration, WIN effect was sensitive to both CB1 and CB2 antagonists in STZ-injected rats (Fig. 4B). However, in ZDF animals, WIN effect was sensitive only to the CB1 antagonist whereas the CB2 antagonist used was unable to block it (Fig. 4B′).

### *3.4. Psychoactive effects of WIN 55212-2 intraperitoneally administered*

In previous works using Wistar rats, we have shown that WIN acutely administered at 1 mg/kg does not exert psychoactive effects (Pascual et al., 2005; Vera et al., 2007). First, we confirmed that this dose did not induce hypolocomotion (which could have interfered with the recording of neuropathic signs) in Wistar rats 20 min after administration: activity counts were 735 ± 57.22 and 663.25 ± 119.50 for WIN- and vehicle-treated animals, respectively ($p > 0.05$), for the whole recording period (30 min), and 199.88 ± 36.18 and 168.88 ± 29.54 for WIN- and vehicle-treated rats, respectively ($p > 0.05$), for the first 5 min of the recording period. Then we confirmed that this dose did not induce analgesia, catalepsy, hypothermia or hypolocomotion in lean rats either (Fig. 5).

As shown above, no significant effect of WIN was detected in the plantar test in diabetic rats (Fig. 5A, A′). Similarly, no catalepsy (Fig. 5B, B′) or hypothermia (Fig. 5C, C′) developed in either model of diabetes after WIN administration. Although a significant reduction in spontaneous locomotor activity was detected in STZ-injected (Fig. 5D) and ZDF (Fig. 5D′) rats vs. their respective controls, WIN did not further decrease this parameter.

## 4. Discussion

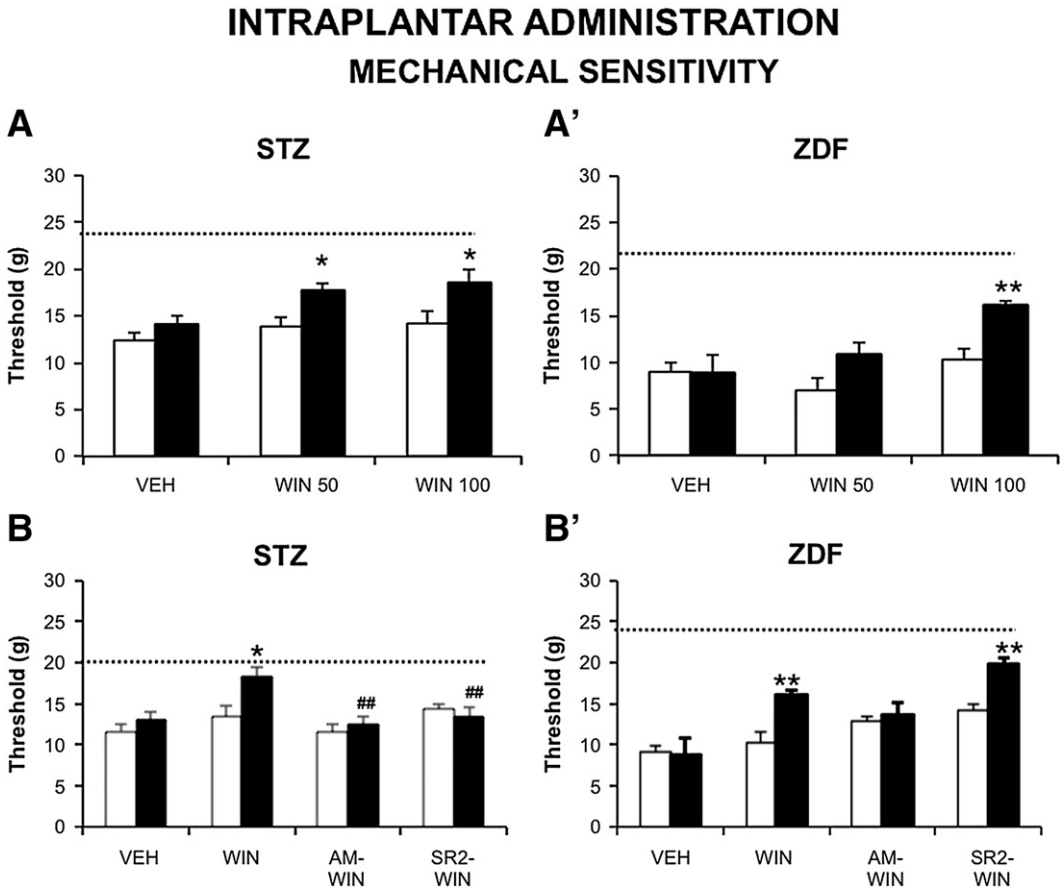
In this work, the non-selective cannabinoid agonist WIN 55,212-2, intraperitoneally administered at a non-psychoactive dose or locally injected, alleviated the signs of peripheral neuropathy in two different models of diabetes mellitus: the streptozotocin-induced model of type 1 diabetes and the Zucker Diabetic Fatty rat model of type 2 diabetes. Unlike in the model of STZ-induced diabetes, in which both cannabinoid receptors seem to play a role, in ZDF rats, the WIN effect probably involves the activation of CB1, but not CB2 receptors. Furthermore, higher doses of WIN were needed to significantly relieve mechanical allodynia upon intraplantar administration in ZDF compared to STZ-injected rats. Our results support the notion that painful neuropathies associated with type 1 and 2 diabetes are not identical and they may need specific treatments.

Despite the common occurrence of diabetic neuropathy, control of pain is one of its most difficult management issues. It often involves different classes of drugs and requires combined therapies. Selection of pain medication and dosage must be individualized; current treatments are only moderately effective and many adverse effects occur (Duby et al., 2004; Vinik et al., 2006). Therefore, alternative therapies are needed.

Cannabinoids are potential analgesics for difficult-to-treat pain (Russo, 2008). In animals, they have been shown to alleviate and/or prevent signs of peripheral neuropathy induced by nerve injury (Herzberg et al., 1997; Malcangio and Tomlinson, 1998; Pascual et al., 2005) or antitumoral drugs (Pascual et al., 2005; Rahn et al., 2007; Vera et al., 2007). Cannabinoids also alleviated neuropathic pain associated with STZ-induced diabetes in both mice (Doğrul et al., 2004; Toth et al., 2010) and rats (Ulugol et al., 2004; Bujalska, 2008). Interestingly, $\Delta^9$-tetrahydrocannabinol (THC) exhibited enhanced antinociceptive efficacy in STZ-diabetic rats, whereas morphine showed reduced antinociceptive efficacy (Williams et al., 2008), suggesting that cannabinoids may show greater therapeutic potential for treating diabetic neuropathy compared to opioids. Therefore, we were interested in studying their effects in a model of type 2 diabetes.

In the present study, the ZDF rat was used as a model of type 2 diabetes, and the rat model of STZ-induced type 1 diabetes was used for comparison. As expected, most STZ-injected rats showed hyperglycemia and mechanical allodynia, but not thermal hyperalgesia (Malcangio and Tomlinson, 1998; Fox et al., 1999; Ulugol et al., 2004). Even though in the present work non-fasting glycemia was only determined on day 21 after diabetes induction, on day 28, when we measured the effect of WIN in neuropathy, hyperglycemia was present in a previous report (Bujalska, 2008). Furthermore, in a parallel study we found glucose levels above 600 mg/dl, 80–90 days after STZ injection (unpublished observations), suggesting that hyperglycemia is a robust feature and may increase throughout time in this model.

Also in agreement with previous reports (Oltman et al., 2005; Li et al., 2006; Brussee et al., 2008), ZDF rats showed higher body weight (at least initially) than lean animals, as well as random hyperglycemia. In addition, they developed tactile allodynia but not thermal

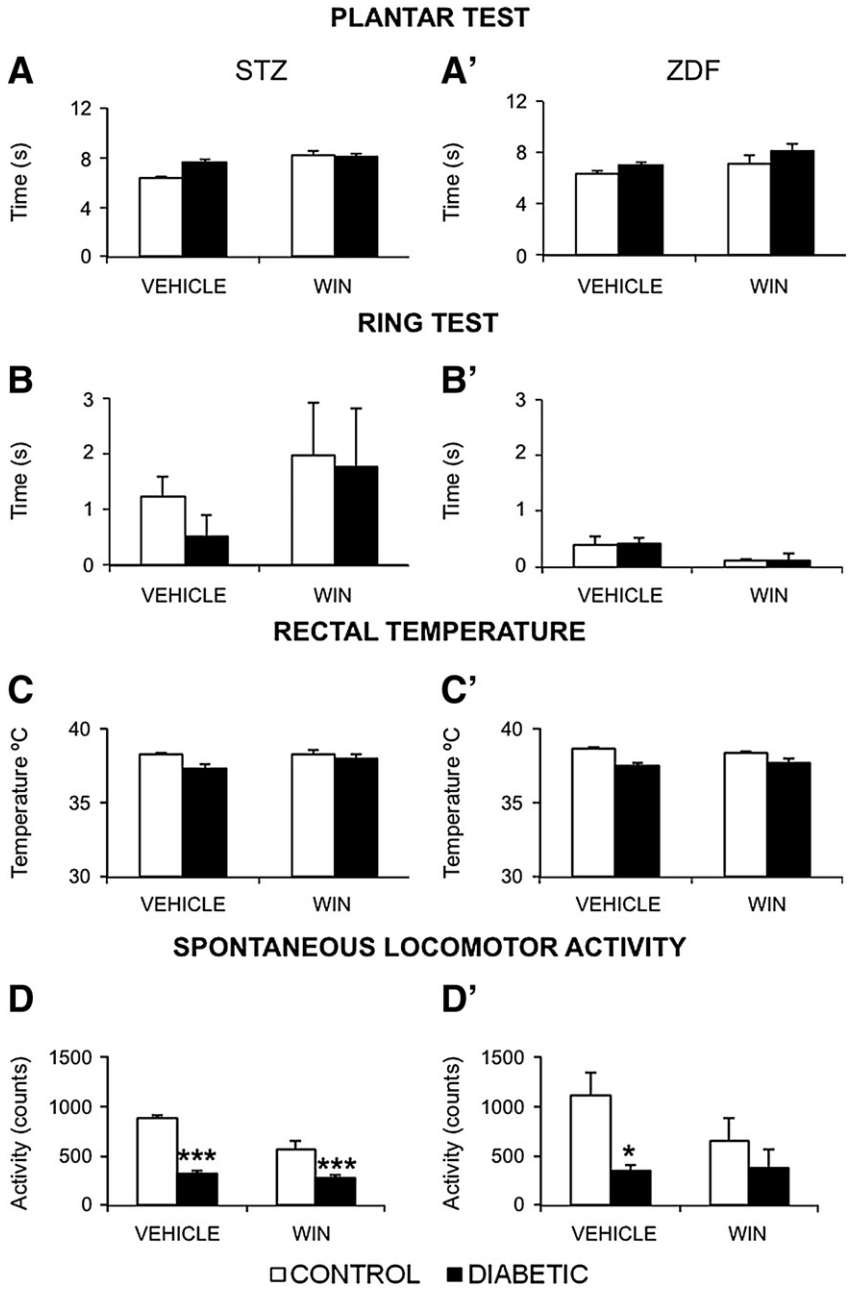



hyperalgesia, which is also consistent with previous studies (Brussee et al., 2008). Moreover, some degree of thermal hypoalgesia was found at the last time point evaluated. Although hypoalgesia has been previously reported, its evaluation was generally carried out at longer time-points (Ohsawa et al., 2008). These duration-dependent changes in the thermal nociceptive threshold probably reflect the symptoms in human diabetic neuropathy. Thermal hyperalgesia is well observed in early stages of diabetes mellitus in human subjects and longer-term of diabetes mellitus shows an increased thermal threshold (Dyck et al., 2000) that can increase the risk of trauma and lead to infection and amputation (Vinik et al., 2006). In any case, mechanical allodynia could be a more robust nociceptive indicator than thermal (heat) hyperalgesia, not only in STZ-treated, but also in ZDF rats (Brussee et al., 2008).

The non-selective cannabinoid agonist WIN completely abolished mechanical allodynia at 1 mg/kg in both STZ-injected and ZDF rats. This dose caused antiallodynic effects in a model of paclitaxel-induced neuropathic pain (Pascual et al., 2005). Although in a previous report much higher doses (at least 3 mg/kg, upon intraperitoneal administration) were needed to reverse mechanical allodynia in STZ-injected diabetic rats (Ulugol et al., 2004), our results on this parameter, and those obtained on mechanical hyperalgesia (Bujalska, 2008) suggest that low, non-psychoactive doses are enough to relieve diabetic neuropathy. The dose of 1 mg/kg was not psychoactive in control Wistar rats (Pascual et al., 2005; Vera et al., 2007), lean rats and type 1 or 2 diabetic rats (present results).

In STZ-treated rats, intraplantar administration of WIN was effective in reducing mechanical allodynia (Ulugol et al., 2004). Our study confirmed these results and extended the notion that locally applied cannabinoids might be useful for relieving neuropathic signs in both type 1 and 2 diabetes, because intraplantar administration of WIN was also effective in ZDF rats. Local administration of WIN significantly reduced mechanical allodynia in the ipsilateral paw, without modifying the threshold in the contralateral paw in type 1 diabetes (present results; Ulugol et al., 2004) and also in type 2 diabetes (present results), suggesting that WIN did not need to reach the CNS to exert an antiallodynic effect at the doses tested (and so, psychoactive effects are minimized).

As a non-selective CB1–CB2 agonist, WIN could exert its antiallodynic effect via CB1 and/or CB2 receptors. Whereas CB1 receptors are expressed primarily within the CNS (Zimmer et al., 1999), CB2 receptors are expressed primarily, but not exclusively, outside the CNS in cells of the immune system (Munro et al., 1993). Furthermore, CB2 receptors are up regulated in the CNS in neuropathic pain states (Wotherspoon et al., 2005; Beltramo et al., 2006). Both CB1- (Herzberg et al., 1997; Fox et al., 2001) and CB2- (Ibrahim et al., 2003; Beltramo et al., 2006) specific mechanisms suppressed neuropathic nociception evoked by traumatic nerve injury. In STZ-induced diabetic mice and rats, both CB1 and CB2 receptors were involved in alleviating mechanical hyperalgesia (Bujalska, 2008), thermal hyperalgesia (Toth et al., 2010) and mechanical allodynia (Toth et al., 2010; present results).




However, in ZDF rats the CB2 antagonist was not capable of blocking the WIN effect. This could at least partially explain why higher intraplantar doses of WIN were required to obtain significant antinociception in ZDF vs. STZ rats. In any case, although confirmatory studies are required, our results support the idea that neuropathy associated with type 1 and 2 diabetes are differentially originated and maintained (Sima, 2008) and that CB2 selective agonists might not be as useful in type 2 diabetes as in other neuropathies including those associated with type 1 diabetes. Interestingly, STZ-induced diabetic rats exhibit a decrease in the expression of CB1R in the dorsal root ganglia (DRG) that contribute to the development and progression of the neurodegeneration found in diabetic animals (Zhang et al., 2007).

The reason why CB2 receptors seem to play a limited role in WIN effect in this particular model of type 2 diabetes remains to be investigated. Nonetheless, because intraplantar administration was effective in the ipsilateral but not in the contralateral paw, it could be speculated that CB1 receptors located at the peripheral nerve endings inside the paw might underlie much (if not all) of the CB1-mediated effect in ZDF rats. It would be interesting to investigate whether CB1 receptors are up regulated in the skin as a means to compensate the decline in DRG. Interestingly, in STZ-injected animals and their controls, the CB1–CB2 agonist CP-55,940 inhibited capsaicin-evoked calcitonin gene related peptide (CGRP) release from the rat paw skin *in vitro* by activating CB1, but not CB2, receptors (Ellington et al., 2002). These results suggest that peripheral CB1 receptors are an interesting

therapeutic target in diabetic neuropathy, irrespective of the underlying etiology. Further work including histological and molecular analysis of both DRG and skin might be helpful to provide a definite answer to this question.

The acute administration of the CB1 and CB2 antagonists alone had no effect in STZ-induced (present results; Toth et al., 2010) or ZDF diabetic rats, suggesting that endocannabinoids might not be tonically released in these models of peripheral neuropathy. However, in a previous study, daily intraperitoneal administration of the CB1 antagonist rimonabant alone reduced mechanical allodynia in diabetic mice (Comelli et al., 2010). More research is needed to clarify whether these differences are due to the pattern of administration, the CB1 antagonist, and/or the animal species used.

Although the cannabinoid agonist WIN did not exert significant psychoactive effects in the cannabinoid tetrad, one parameter was different in diabetic vs. control animals. Both STZ-injected and ZDF rats showed lower basal spontaneous locomotor activity than their controls (vehicle-treated and lean rats, respectively). Several factors may contribute to this reduced activity, including neuropathy and alterations in bone and skeletal muscle mass and function. Indeed, STZ-induced diabetes attenuates muscle growth (Armstrong and Laughlin, 1985; Price et al., 1996) and alters bone structure, leading to poor mechanical properties (Einhorn et al., 1988). Similarly, low basal activity in ZDF rats might be related to their altered bone mass and mechanical properties (Prisby et al., 2008).

## 5. Conclusions

Cannabinoids may exert an antineuropathic effect in both type 1 and type 2 diabetes, which is achieved at low, non-psychoactive doses, by systemic or local administration. However, cannabinoid CB1 and CB2 receptors seem to be differentially involved in the cannabinoid antineuropathic effect: whereas in STZ-induced (type 1) diabetes, both CB1 and CB2 receptors seem to be equally involved, in ZDF rats (type 2 diabetes), CB1 receptors might play a more significant role. Thus, cannabinoids, probably mainly through the activation of peripheral CB1 receptors, may constitute a new therapeutic alternative in painful neuropathy associated with type 2 diabetes. Our results support the notion that in order to evaluate a novel drug with potential activity in diabetic neuropathy it is essential to adequately select the animal model.

## Competing interests

The authors declare that they have no competing interests.

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