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Celecoxib reduces hyperalgesia and tactile allodynia in diabetic rats

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ABSTRACT

Background: In the present study we determined the antihyperalgesic and antiallodynic effect of celecoxib in diabetic rats as well as the possible participation of opioid receptors in the mechanism of action of celecoxib in these rats.
Methods: Experimental diabetes was induced by streptozotocin. Formalin (0.5%) was used to produce hyperalgesia in non-diabetic and diabetic rats. von Frey filaments were used to determine the 50% withdrawal threshold in diabetic rats.
Results: Oral administration of celecoxib (0.3–30 mg/kg) reduced formalin-induced nociceptive behavior during phase 2. Systemic pre-treatment (−10 min) with naltrexone (3 mg/kg) prevented celecoxib-induced antihyperalgesia in formalin-treated diabetic rats. Furthermore, naltrexone as well as the δ and κ opioid receptor antagonists naltrindole (3 mg/kg) and 5′-guanidino naltrindole (1 mg/kg), respectively, fully prevented celecoxib-induced antihyperalgesia (10 mg/kg) in formalin-treated non-diabetic and diabetic rats. Furthermore, celecoxib (0.3–30 mg/kg) produced an antiallodynic effect in diabetic rats. Pre-treatment with naltrexone (3 mg/kg) fully prevented the antiallodynic effect of celecoxib at 0.3, 3 and 10 mg/kg. In contrast, this dose of naltrexone only partially prevented the antiallodynic effect of celecoxib 30 mg/kg. Naltrexone and naltrindole (3 mg/kg), but not 5′-guanidino naltrindole (1 mg/kg), fully prevented the antiallodynic effect of celecoxib in diabetic rats.
Conclusions: Data suggest that celecoxib produces an antihyperalgesic and antiallodynic effect in diabetic rats. These effects seem to result from activation of μ, δ and κ opioid receptors for antinociception and μ and δ for antiallodynia. Celecoxib could be useful to treat neuropathic pain in diabetic patients.
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Introduction

Diabetes mellitus is one of the most common chronic medical conditions affecting over 100 million people world-wide, of whom up to 50% may develop diabetic neuropathy [1]. The treatment of pain in diabetic patients is frequently unsatisfactory. Anticonvulsants, tricyclic antidepressants and opioids have become the mainstay in the treatment of chronic neuropathic pain [2,3]. However, these drugs often have a limited effect or they may cause intolerable side effects. Therefore, other options of treatment are needed.

The definitive role of prostanoids in neuropathic pain is still a matter of debate. Several studies have shown the usefulness of acute or repetitive administration of non-steroidal anti-inflammatory drugs (NSAIDs) [4–8], when they are given before or immediately after nerve injury. In contrast, other studies have found that NSAIDs do not reverse established neuropathic pain in rats [9–11]. Taken together, these data point that prostanoids play an important role during development but not maintenance of neuropathic pain. However, recent evidence suggests that prostaglandin

Abbreviations: ANOVA, analysis of variance; COX-2, cyclo-oxygenase 2; NSAIDs, non-steroidal anti-inflammatory drugs.

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synthesis *via* cyclo-oxygenase 2 (COX-2) may contribute to the maintenance of neurophatic pain as this protein is up-regulated in the spinal cord and periphery after nerve injury [12–15]. In agreement with this idea, intrathecal administration of the COX-2 inhibitors GW406381, celecoxib and etodolac have shown to reduce tactile allodynia in neuropathic rats [16–19]. Furthermore, the preferential COX-2 inhibitor meloxicam as well as the selective COX-2 inhibitors SC-58125 and NS-398 are able to diminish established hypersensitivity in diabetic rats [15,20–22].

Celecoxib exhibits anti-pyretic, anti-inflammatory and analgesic activities [23] attributed to the inhibition of prostaglandin synthesis [24,25]. However, other mechanisms [19,26] including endogenous opioids [26–28] have been proposed for this drug. The effects of celecoxib in diabetic pain have been scarcely studied [19,29,30]. Thus, the purpose of this study was to assess the antihyperalgesic and antiallodynic effects of celecoxib in diabetic rats. Furthermore, the possible participation of opioid receptors in the antihyperalgesic and antiallodynic effect of celelecoxib in diabetic rats was also determined.

Material and methods

Animals

Experiments were performed on adult male Wistar rats (body weight range, 230–250 g) of 9–10 weeks of age. Rats were obtained from the Facultad de Medicina, UNAM (México City). The animals were housed and maintained at 22 ± 2 °C under a 12-h light/12-h dark cycle with free access to food and water. Experiments were started at the same time (10:00 AM). Efforts were made to minimize animal suffering and to reduce the number of animals used. All the experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [31] and were approved by our local Ethics Committee (DACS, UJAT).

Induction of diabetes

Rats were injected with streptozotocin (60 mg/kg, *ip*) (Sigma, St. Louis, MO, USA) to produce experimental diabetes [32]. Control animals (age-matched) received distilled water. Diabetes was confirmed 4 days after injection by measurement of tail vein blood glucose levels with the Accu-Check Sensor Comfort glucometer (Roche, Mexico City). Four weeks after streptozotocin injection, glycemia was again determined and only animals with a final blood glucose level ≥250 mg/dl were included in the study. Experiments were started with numbers greater than six considering that only 80–90% of the streptozotocin-treated rats became hyperglycemic or survived at two weeks. Thus, groups had to be started considering this fact.

Assessment of hyperalgesia

Hyperalgesia in non-diabetic and diabetic (four weeks) rats was assessed using the 0.5% formalin test [33,34]. The rats were placed in open plexiglas observation chamber for 30 min to acclimatize to their surroundings; then were removed for formalin administration. Fifty microliters of diluted formalin (0.5%) were injected subcutaneously into the dorsal surface of the right hind paw with a 30-ga needle. The animals were returned to the chambers and nociceptive behavior was observed immediately after formalin injection. Mirrors were placed in each chamber to enable unhindered observation. Nociceptive behavior was quantified as the numbers of flinches of the injected paw during 1 min periods every 5 min, up to 60 min after injection [33,35]. Flinching was readily discriminated and was characterized as a rapid and brief withdrawal or as a flexing of the injected paw. Formalin induced flinching behavior was biphasic [35]. The initial acute phase (0–10 min) was followed by a relatively short quiescent period, which was then followed by a prolonged tonic response (10–60 min). Animals were used only once and at the end of the experiment they were sacrificed in a CO_2 chamber.

Assessment of allodynia

Tactile allodynia was tested in diabetic rats 4 weeks after streptozotocin injection as previously reported [36]. Rats were transferred to a clear plastic, wire mesh-bottomed cage and allowed to acclimatize for 30 min. Von Frey filaments (Stoelting, Wood Dale, IL, USA) were used to determine the 50% paw withdrawal threshold using the up-down method of Dixon [37]. A series of filaments, starting with one that had a buckling of 2 g, was applied in consecutive sequence to the plantar surface of the right hind paw with a pressure causing the filament to buckle. Lifting of the paw indicated a positive response and prompted the use of the next weaker filament whereas the absence of a paw withdrawal after 5 s indicated a negative response and prompted the use of the next filament of increasing weight. This paradigm continued until four more measurements had been made after the initial change of the behavioral response or until 5 consecutive negative (assigned a score of 15 g) or four consecutive positive (assigned a score of 0.25 g) responses had occurred. The resulting scores were used to calculate the 50% response threshold by using the formula: 50% g threshold = $10^{(Xf+\kappa\partial)}/10{,}000$. Where Xf = the value (in log units) the final von Frey filament used [36], κ = the value for the pattern of positive and/or negative responses, and ∂ = the mean difference (in log units) between stimulus strengths.

Withdrawal threshold assessment was performed immediately before and every 30 min until 3.5 h after drug administration. Allodynia was considered to be present when paw withdrawal thresholds were <4 g. Diabetic rats not demonstrating allodynia were not further studied.

Drugs

Streptozotocin, naltrexone, naltrindole and 5′-guanidino naltrindole were purchased from Sigma (St. Louis, MO, USA). Celecoxib was obtained from Pfizer, S.A. de C.V. (Mexico City, Mexico). Streptozotocin was freshly dissolved in distilled water, protected from light and immediately administered. Naltrexone, naltrindole and 5′-guanidino naltrindole were dissolved in 0.9% isotonic saline, while celecoxib was dissolved in 40% polyethylene glycol.

Study design

Independent groups of animals were used for each experimental condition. Dose–response curve for administration of celecoxib was carried out giving vehicle or increasing doses of celecoxib (0.3–30 mg/kg) 30 min before formalin injection into the right paw.

To determine the possible participation of the opioid system in the antihyperalgesic activity of celecoxib in diabetic rats, naltrexone (a non-selective opioid receptor antagonist, 3 mg/kg) was administered in combination with increasing doses of celecoxib (0.3–30 mg/kg). Furthermore, naltrexone (3 mg/kg), naltrindole (a δ opioid receptor antagonist, 3 mg/kg) or 5′-guanidino naltrindole (a κ opioid receptor antagonist, 1 mg/kg) was administered 10 min before a fixed dose of celecoxib (10 mg/kg), which was given 30 min before formalin injection, and the formalin-induced nociceptive behavior was assessed.

For the study of allodynia, rats received an oral administration of vehicle (300 μl; 40% polyethylene glycol) or increasing doses of celecoxib (0.3–30 mg/kg) and withdrawal threshold in non-diabetic and diabetic (4 weeks) rats was measured for the next

3.5 h. To determine the possible participation of the opioid system in celecoxib-induced antiallodynic effect in diabetic rats, naltrexone (3 mg/kg) was administered in combination with increasing doses of celecoxib (0.3–30 mg/kg). To explore the participation of other opioid receptors, diabetic rats received a bolus injection of naltrexone (3 mg/kg), naltrindole (3 mg/kg) or 5′-guanidinonaltrindole (1 mg/kg) 10 min before injection of a fixed dose of celecoxib (10 mg/kg) and the withdrawal threshold was assessed.

Observer was unaware of the treatment in each animal. The doses and the drug administration schedule of celecoxib, naltrexone, naltrindole and 5′-guanidino naltrindole were selected based on previous reports [38,39] and on pilot experiments in our laboratory conditions.

Data analysis and statistics

All results are presented as the mean ± SEM of 6 animals per group. The curves were constructed by plotting the 50% withdrawal threshold as a function of time. From these plots, area under the 50% threshold withdrawal against time curve (AUC) was computed. For the formalin test, the nociceptive behavior induced by the *sc* injection of 0.5% formalin in non-diabetic and diabetic rats was registered as the numbers of flinches of the injected paw during 1 min periods every 5 min, up to 60 min after injection. In addition, the sum of flinches was used to have a global measure of nociception, as previously reported [15].

One- or two-way analysis of variance (ANOVA) followed by Tukey's test was used to compare differences between more than 2 treatments. Differences were considered to reach statistical significance when $p < 0.05$.

Results

Formalin-evoked flinching behavior in non-diabetic and diabetic rats

Streptozotocin injection provoked hyperglycemia. The blood glucose levels measured in these rats before (92.4 ± 0.3 mg/dl) and after streptozotocin (432.2 ± 17.6 mg/dl) injection singnificantly increased whereas that those in rats treated with saline did not change (93.4 ± 0.7 *vs.* 91.1 ± 0.4 mg/dl). Diabetic rats also showed polyuria and increased food and water intake as previously reported [22,33,38].

Diabetic (4 weeks) and non-diabetic animals subjected to 0.5% formalin showed a biphasic time-course as described elsewhere [15] (Fig. 1A). Diabetic rats showed an increased sum of flinches ($p < 0.05$), compared to non-diabetic rats (Fig. 1B), which confirm the enhanced hypersensitivity reported in diabetic animals [15,33,34].

Antihyperalgesic effect of celecoxib in non-diabetic and diabetic rats

Pre-treatement (−30 min) with celecoxib (0.3–30 mg/kg, *po*) significantly ($p < 0.05$) diminished formalin-induced nociceptive behavior during phase 2 in a dose-dependent manner in non-diabetic (Fig. 2A, Fig. S1) and 4 weeks diabetic rats (Fig. 2B, Fig. S2). Insets in panels A and B show the the time-course for the highest dose of celecoxib tested. In contrast, celecoxib did not affect phase 1 of the formalin test in non-diabetic and diabetic rats (Inset in Fig. 2, Figs. S1–S2).

Effect of opioid receptor antagonists on celecoxib-induced antihyperalgesia in diabetic rats

Intraperitoneal pre-treatment (−10 min) with the non-selective opioid receptor antagonist naltrexone (3 mg/kg) prevented celecoxib-induced antihyperalgesia in all doses during phase 2 of formalin test in 4 weeks diabetic rats (Fig. 3, Fig. S3). Systemic administration of naltrexone *per se* did not affect formalin-induced flinching behavior in 4 weeks diabetic rats (Fig. 3). Furthermore, naltrexone as well as the selective δ and κ opioid receptor antagonists naltrindole (3 mg/kg) and 5′-guanidinonaltrindole (1 mg/kg), respectively, prevented ($p < 0.05$) the antihyperalgesic effect of celecoxib (10 mg/kg) in both non-diabetic (Fig. 4A) and 4 weeks diabetic rats (Fig. 4B).

Antiallodynic effect of celecoxib in diabetic rats

Streptozotocin, but not saline, injection produced tactile allodynia 4 weeks after injection (Fig. 5). In these diabetic rats, oral administration of celecoxib (30 mg/kg), but not vehicle, produced an antiallodynic effect (Inset Fig. 6). Furthermore, this COX-2 inhibitor dose-dependently enhanced ($p < 0.05$) the 50% withdrawal threshold indicating an antiallodynic effect (Fig. 6, Fig. S4). In support of our data, other COX-2 inhibitor, meloxicam,

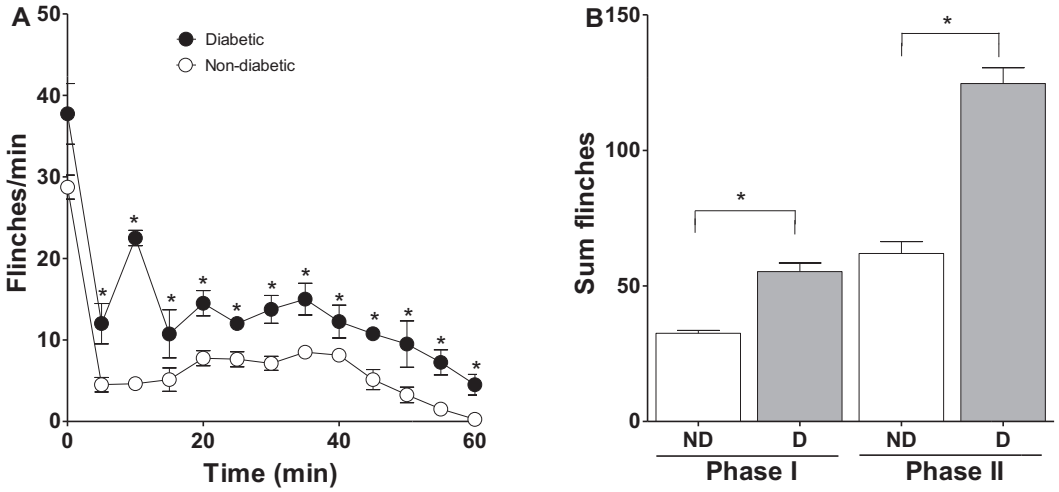

Fig. 1. Time course of the nociceptive behavior induced by the subcutaneous injection of 0.5% formalin to non-diabetic (white circles) and diabetic (black circles) rats (A). Sum of flinches counted during phase I and phase II in non-diabetic and diabetic rats subjected to the formalin test (B). Data are the mean ± SEM of 6 animals. * Significantly different from the non-diabetic group ($p < 0.05$), as determined by two-way ANOVA followed by the Student–Newman–Keuls test.

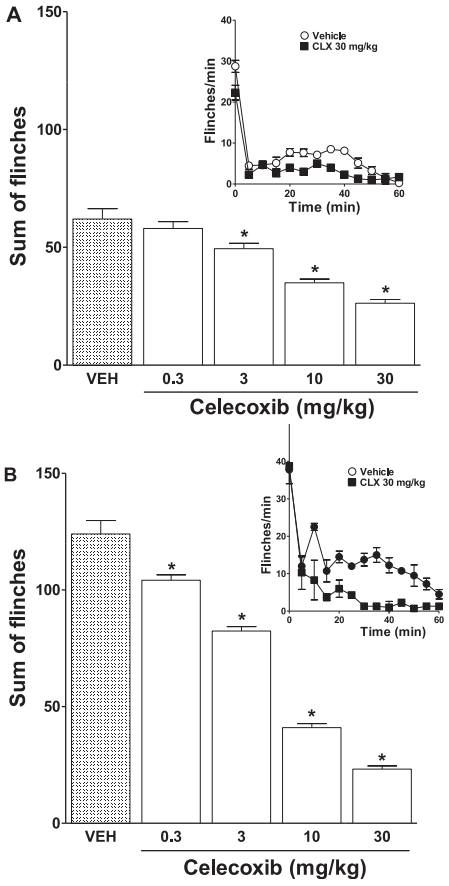

Fig. 2. Dose–response curves of celecoxib (0.3–30 mg/kg, *ip*) in non-diabetic (A) and diabetic (B) rats during phase 2 of the formalin test. Inset, in both plots, shows time course of the antihyperalgesic effect for the highest dose of celecoxib used (30 mg/kg). Data are expressed as the sum of flinches. Data are expressed as mean ± SEM of 6 rats. * Significantly different from the vehicle (VEH) group ($p < 0.05$), by one-way ANOVA followed by the Student–Newman–Keuls test.

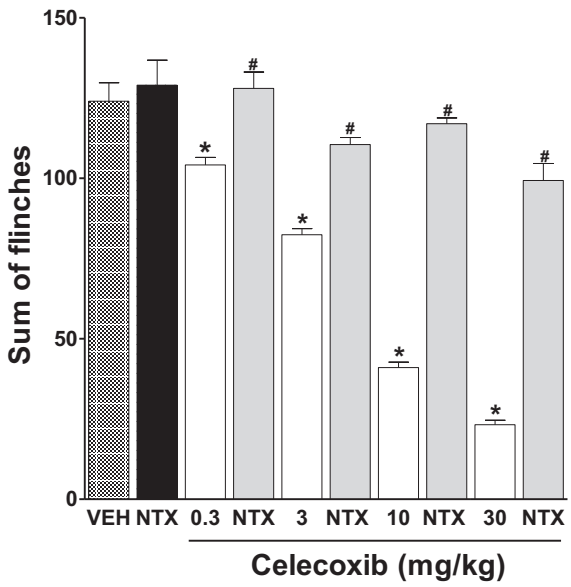

Fig. 3. Effect of naltrexone (NTX, 3 mg/kg, *ip*) on celecoxib (0.3–30 mg/kg, *ip*)-induced antinociceptive activity in diabetic rats during phase 2 of the formalin test. Data are expressed as the sum of flinches. Data are the mean ± SEM of 6 rats. * Significantly different from the vehicle (VEH) group ($p < 0.05$) and # significantly different from the celecoxib group ($p < 0.05$), by two-way ANOVA followed by the Student–Newman–Keuls test.

was able to diminish tactile allodynia in 4 weeks diabetic rats (Fig. S5).

Effect of opioid receptor antagonists on celecoxib-induced antiallodynia in diabetic rats

Intraperitoneal pre-treatment (−10 min) with the non-selective opioid receptor antagonist naltrexone (3 mg/kg) fully prevented the antiallodynic effect of celecoxib 0.3, 3 and 10 mg/kg in 4 weeks diabetic rats (Fig. 7, Fig. S6). In marked contrast, this dose of naltrexone only partially prevented the antiallodynic effect of celecoxib 30 mg/kg in 4 weeks diabetic rats (Fig. 7, Fig. S6). In addition, naltrexone as well as the selective δ opioid receptor

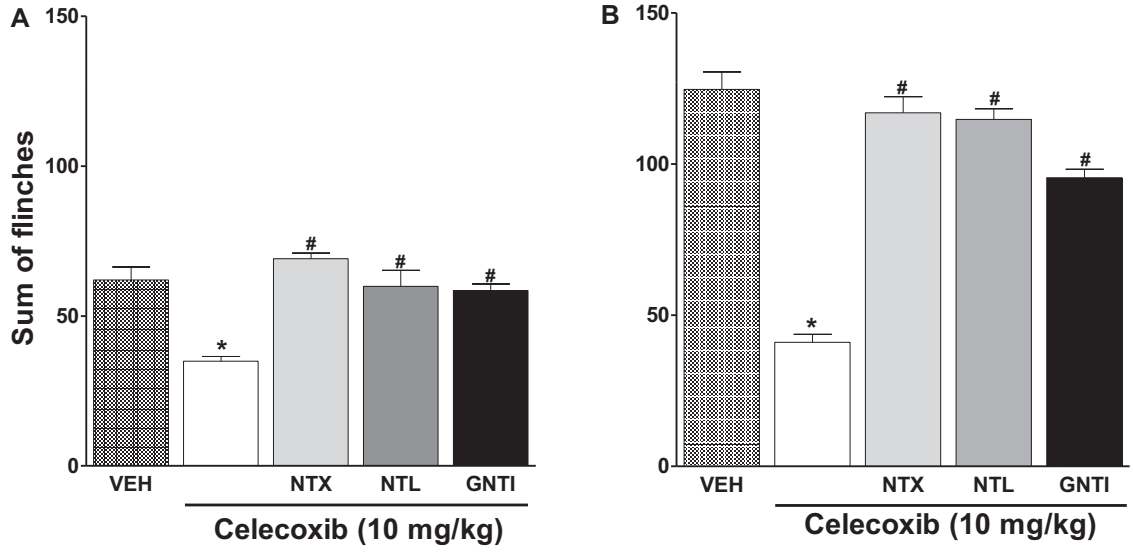

Fig. 4. Effect of naltrexone (NTX, 3 mg/kg, *ip*), naltrindole (NLT, 3 mg/kg, *ip*) or 5-guanidinonaltrindole (GNTI, 1 mg/kg, *ip*) on celecoxib (10 mg/kg, *ip*)-induced antinociceptive activity in non-diabetic (A) and diabetic (B) rats during phase 2 of the formalin test. Data are expressed as the sum of flinches. Data are the mean ± SEM of 6 rats. * Significantly different from the vehicle (VEH) group ($p < 0.05$) and # significantly different from the celecoxib group ($p < 0.05$), by one-way ANOVA followed by the Student–Newman–Keuls test.

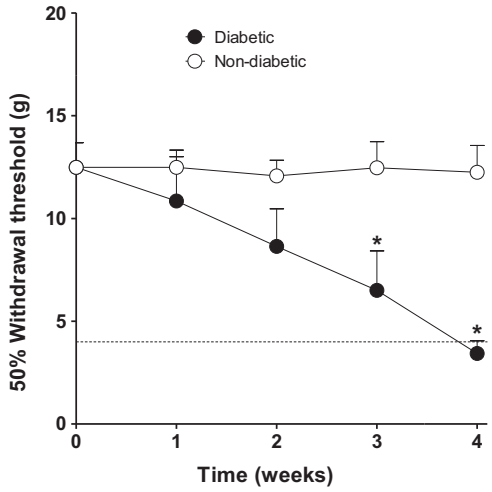

Fig. 5. Time course of tactile allodynia observed in streptozotocin-pretreated rats. After streptozotocin injection, animals were allowed to develop tactile allodynia for 4 weeks (black circles). Control animals (no diabetic) had a normal sensitivity (white circles). Data are expressed as the 50% withdrawal threshold (mean ± SEM) of 6 rats. * Significantly different from the non-diabetic group ($p < 0.05$), by two-way ANOVA followed by the Student–Newman–Keuls test.

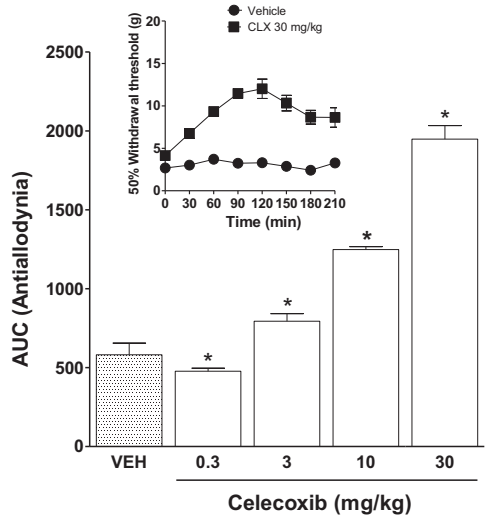

Fig. 6. Dose–response curve of celecoxib in diabetic rats. Four weeks after streptozotocin injection, animals were treated with vehicle or celecoxib (0.3–30 mg/kg, *ip*). Inset shows time course for the highest dose of celecoxib used (30 mg/kg). Data are presented as the area under the 50% withdrawal threshold against time curve (AUC). Data are the mean ± SEM of 6 rats. * Significantly different from vehicle (VEH) group ($p < 0.05$), by one-way ANOVA followed by the Student–Newman–Keuls test.

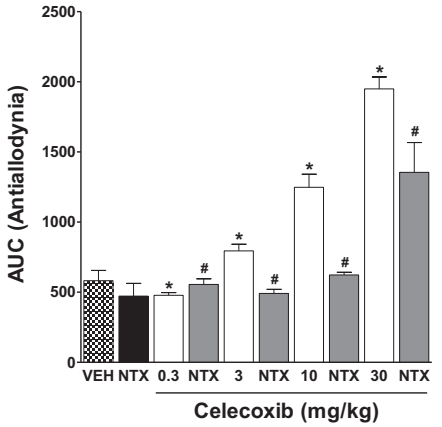

Fig. 7. Effect of naltrexone (NTX, 3 mg/kg, *ip*) on celecoxib (0.3–30 mg/kg, *ip*)-induced antiallodynic activity in 4 weeks diabetic rats. Data are expressed as the area under the 50% withdrawal threshold against time curve (AUC). Data are the mean ± SEM of 6 rats. * Significantly different from the vehicle (VEH) group ($p < 0.05$) and # significantly different from the celecoxib group ($p < 0.05$), by two-way ANOVA followed by the Student–Newman–Keuls test.

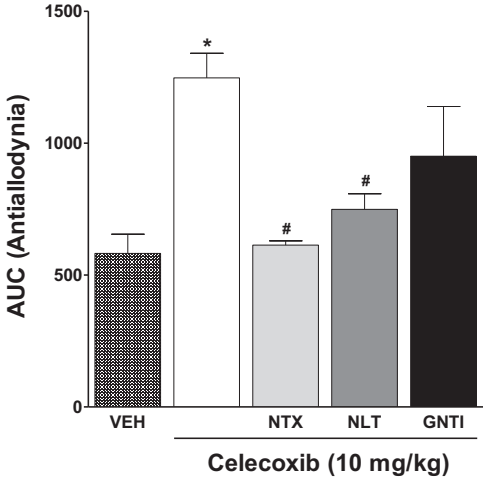

Fig. 8. Effect of naltrexone (NTX, 3 mg/kg, *ip*), naltrindole (NLT, 3 mg/kg, *ip*) or 5-guanidinonaltrindole (GNTI, 1 mg/kg, *ip*) on celecoxib (10 mg/kg, *ip*)-induced antiallodynic activity in diabetic rats. Data are expressed as the area under the 50% withdrawal threshold against time curve (AUC). Data are the mean ± SEM of 6 rats. * Significantly different from the vehicle (VEH) group ($p < 0.05$) and # significantly different from the celecoxib group ($p < 0.05$), by one-way ANOVA followed by the Student–Newman–Keuls test.

antagonist naltrindole (3 mg/kg), but not the selective κ opioid receptor antagonist 5′-guanidino naltrindole (1 mg/kg), fully prevented ($p < 0.05$) the antiallodynic effect of celecoxib (10 mg/kg) in 4 weeks diabetic rats (Fig. 8).

Discussion

Antihyperalgesic and antiallodynic effect of celecoxib in non-diabetic and diabetic rats

In the present study, we observed that systemic administration of celecoxib was able to prevent formalin-induced flinching behavior during phase 2 in non-diabetic and 4 weeks diabetic rats subjected to noxious stimulation with 0.5% formalin. These data agree with previous studies showing that systemic celecoxib diminishes formalin- or carrageenan-induced hyperalgesia in non-diabetic rats and mice [40–45]. Furthermore, our study also agree with data showing that celecoxib reduces hyperalgesia in diabetic rats [12,15,20,29]. In contrast, others have reported that subcutaneous injection of celecoxib (10 mg/kg) does not diminish hyperalgesia in Sprague-Dawley rats 5 days after streptozotocin [30]. Discrepancy could be due to the dose of celecoxib (10 *vs.* 30 mg/kg) or time to assess hyperalgesia or allodynia (5 days *vs.* 4 weeks). Besides the antihyperalgesic effect of celecoxib in non-diabetic and diabetic rats, we found that celecoxib and meloxicam were able to reverse streptozotocin-induced tactile allodynia in 4 weeks diabetic rats. As far as we can determine, it is the first report showing that celecoxib and meloxicam diminish tactile allodynia in diabetic rats. However, there is evidence that celecoxib reduces tactile allodynia in rats subjected to the brachial plexus avulsion or chronic constriction injury [19,46]. Thus, it seems that

celecoxib reduces hyperalgesia and allodynia in diabetic and neuropathic rats. Since celecoxib is a selective COX-2 inhibitor [25,47], our data suggest that COX-2 may have a participation in streptozotocin-induced hypersensitivity. Regarding this, there is evidence that COX-2 expression is up-regulated in the spinal cord of rats subjected to painful diabetic neuropathy while intrathecal injection of COX inhibitors relieves nociception in those animals [12,15,20,48]. However, some studies have reported that celecoxib and meloxicam may modulate potassium [19,49,50] and sodium [51–53] channels in a COX-2 independent manner. It is currently accepted that blockade of sodium channels [54–56] as well as the activation of potassium channels [19,56] leads to inhibition of tactile allodynia in neuropathic pain models. Other studies have suggested that intraplantar or *icv* celecoxib may indirectly stimulate opioid receptors [26,28]. Thus, it is likely that besides COX-2 inhibition other mechanisms could be pivotal for the antinociceptive effect of celecoxib in diabetic rats in the present study.

Effect of opioid receptor antagonists in celecoxib-induced antinociception

We found that the non-selective opioid receptor antagonist naltrexone diminished the anthyperalgesic and antiallodynic effect of celecoxib in non-diabetic and diabetic rats suggesting that the anthyperalgesic activity of celecoxib in diabetic rats involves activation of opioid receptors. Our data agree with previous studies showing a naltrexone-sensitive hypoalgesic effect of celecoxib in non-diabetic rats [27,41]. Furthermore, the selective δ and κ opioid receptor antagonists naltrindole and 5′-guanidino naltrindole, respectively, prevented celecoxib-induced antihyperalgesic effects in diabetic rats. These data imply that celecoxib may activate μ, δ and κ opioid receptors to produce antihyperalgesia in diabetic rats. Similar observations have been reported in non-diabetic rats [26,28]. The fact that bestatin, an inhibitor of the metabolism of endogenous opioid peptides, increases celecoxib effect in non-diabetic rats [27] further reinforces our suggestion.

It is worthy to state that naltrexone was able to fully prevent the antihyperalgesic effect of all doses of celecoxib. In contrast, this antagonist fully prevented the antiallodynic effect of all doses of celecoxib (0.3–10 mg/kg) with exception of the greatest dose used (30 mg/kg). These results suggest that the anthyperalgesic effects of celecoxib in diabetic rats depend on the activation of opioid receptors while the antiallodynic effect of this drug (at the greatest dose) results from activation of opioid receptors plus other mechanisms. Interestingly, naltrexone and naltrindole fully prevented the antihyperalgesic effect of celecoxib (10 mg/kg) while 5′-guanidino naltrindole partially prevented this effect suggesting the participation of μ and δ, and at lesser extent κ, opioid receptors. In marked contrast, naltrexone and naltrindole fully prevented the antiallodynic activity of celecoxib while 5′-guanidino naltrindole did not affect such effect suggesting the participation of μ and δ, but not κ, opioid receptors. The mechanisms of how celecoxib could activate opioid receptors are at present unknown. It seems that celecoxib does not directly bind opioid receptors, even though opioid receptor antagonists blocked their effects. Thus, it is likely that celecoxib may release endogenous opioids, which in turn could activate opioid receptors [27] to produce antinociception. Regarding the site of action for celecoxib, there is evidence that intrathecal injection of COX inhibitors relieves nociception in diabetic animals [12,15,20,48]. Thus, the opioid receptors present in the dorsal horn could the target of celecoxib in this study.

Recent studies have pointed that the main mechanism responsible for the antiallodynic effects of celecoxib may not be related to COX-2. This idea is based on the fact that (1) Selective COX-2 inhibitors does not reverse established neuropathic pain in rats [7,9,11,57,58]; (2) Celecoxib activates Kv7/M K^+ channels and reduces tactile allodynia in diabetic and neuropathic rats with a potency and efficacy which is inversely related to their COX-2 inhibitory activity [19]; (3) Celecoxib activates the cannabinoid CB1 receptors [26]; and (4) Celecoxib blockades sodium channels [51–53,58] and this effect has been related to inhibition of tactile allodynia [59]. Thus, activation of potassium channels, blockade of sodium channels, activation of opioid receptors (this study; [27]) or a combination of these could be a more likely mechanism of action for this drug in diabetic rats.

Final considerations and limitations

The relevance of formalin-induced hyperalgesia and allodynia to the painful diabetic neuropathy in humans is unclear. However, the fact that formalin injection leads to peripheral and central hypersensitivity [60] suggest that formalin-induced hyperalgesia and allodynia in diabetic animals could have a correlation with the peripheral hyperexcitability that promotes pain in some diabetic patients [61,62].

Our data suggest that, besides μ and δ opioid receptors, celecoxib could stimulate Kv7/M K^+ channels to produce its antiallodynic effect. A limitation of our study was not to assess the possible participation of Kv7/M K^+ channels in diabetic rats. Inhibition of COX-2 to explain the antiallodynic effect of celecoxib is unlikely as other COX-2 inhibitors are unable to reduce established neuropathic pain in rats [63,64] although this has been disputed [14].

In conclusion, our results suggest that the antihyperalgesic and antiallodynic activity of celecoxib in diabetic rats involve opioid receptors.

Conflict of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pharep.2014.12.006.

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