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Original research article

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.

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