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European Journal of Pharmacology 530 (2006) 48–53
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Benfotiamine relieves inflammatory and neuropathic pain in rats

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Received 27 July 2005; received in revised form 25 October 2005; accepted 4 November 2005
Available online 15 December 2005

Abstract

Benfotiamine has shown therapeutic efficacy in the treatment of painful diabetic neuropathy in human beings. However, so far there is no evidence about the efficacy of this drug in preclinical models of pain. The purpose of this study was to assess the possible antinociceptive and antiallodynic effect of benfotiamine in inflammatory and neuropathic pain models in the rat. Inflammatory pain was induced by injection of formalin in non-diabetic and diabetic (2 weeks) rats. Reduction of flinching behavior was considered as antinociception. Neuropathic pain was induced by either ligation of left L5/L6 spinal nerves or administration of streptozotocin (50 mg/kg, i.p.) in Wistar rats. Benfotiamine significantly reduced inflammatory (10–300 mg/kg) and neuropathic (75–300 mg/kg) nociception in non-diabetic and diabetic rats. Results indicate that oral administration of benfotiamine is able to reduce tactile allodynia from different origin in the rat and they suggest the use of this drug to reduce inflammatory and neuropathic pain in humans.

Keywords: Benfotiamine; Inflammatory pain; Neuropathy; Diabetes

1. Introduction

Neuropathic pain represents a chronic pain syndrome group with diverse etiology, but that shares some common underlying pathophysiologic mechanisms and clinical features. The clinical features of neuropathic pain include the paradox combination of sensory loss in the painful neuropathic area and hypersensitivity phenomena such as touch-evoked pain (allodynia) in the same area. The allodynia is assumed to reflect a neuronal hyperexcitability in the central nervous system (Koltzenburg et al., 1992).

Anticonvulsants and tricyclic antidepressants have become the mainstay in the treatment of chronic neuropathic pain (McQuay et al., 1996; Sindrup and Jensen, 1999). However, these drugs often have a limited effect and they may cause intolerable side effects. Therefore, other options of treatment have been explored. B vitamins have been proposed as possible drugs to treat neuropathic pain. In this sense, previous evidence showed that thiamine and pyridoxine are useful in the treatment of symptomatic diabetic peripheral neuropathy (Abbas and Swai, 1997). A recent study found evidence that the combination of thiamine, pyridoxine and cyanocobalamin improves the analgesic effect of gabapentin in the treatment of diabetic neuropathy (Medina-Santillán et al., 2004). Then, vitamins could be an alternative to treat neuropathic pain.

Benfotiamine was synthesized in early 1960s as a thiamine (vitamin B_1) derivative with high bioavailability (Fujiwara, 1954; Bitsch et al., 1991). Previous studies have showed that thiamine or the mixture of thiamine, pyridoxine and cyanocobalamin could have a role in the treatment of painful neuropathy (Stracke et al., 1996); however, since these vitamins are water-soluble, the rate of absorption was relatively small. Benfotiamine is a lipid-soluble analogue of vitamin B_1 with capacity to

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doi:10.1016/j.ejphar.2005.11.016

reach several organs in animals and humans (Woelk et al., 1998). This drug has shown therapeutic efficacy in the treatment of alcoholic polyneuropathy (Woelk et al., 1998) and painful diabetic neuropathy (Winkler et al., 1999) in human beings. However, so far there is no evidence about the efficacy of benfotiamine in preclinical models of neuropathic pain. B vitamins have also shown to reduce inflammatory pain in animals (França et al., 2001; Reyes-García et al., 2001). Therefore, the purpose of this study was to assess the possible antiallodynic and antinociceptive effect of benfotiamine in neuropathic and inflammatory pain, respectively, models in the rat.

2. Material and methods

2.1. Animals

Experiments were performed on adult female Wistar rats. Female rats were used based on the fact that previous experiments in our conditions (Wistar rats, formalin concentration 1% and weight range 180–220 g) have not shown significant differences between males and females (unpublished data). Other authors have found differences only with other rat strains, greater weight or different formalin concentrations (Gaumond et al., 2002). The animals were obtained from our own breeding facilities and had free access to drinking water, but food was withdrawn 12 h before experiments. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). Additionally, the study was approved by the Institutional Animal Care and Use Committee (Centro de Investigación y de Estudios Avanzados, México, DF, México).

2.2. Induction of diabetes

Rats (weight range, 220–240 g) were intraperitoneally (i.p.) injected with streptozotocin (50 mg/kg; Research Biochemical International, USA) to produce experimental diabetes (Courteix et al., 1993). Control animals (age-matched) received saline 0.9%. Diabetes was confirmed 1 week after injection by measurement of tail vein blood glucose levels with the glucose meter Ascensia ELITE (Bayer, Mexico City). Diabetic rats were divided in two groups, one of 2 weeks (hyperalgesia) and other of 4 to 6 weeks (allodynia) after injection of streptozotocin. Glycemia was determined and only animals with a final blood glucose level $\geq$300 mg/dl were included in the study.

2.3. Measurement of antinociceptive activity

Antinociception in non-diabetic and diabetic (2 weeks) rats was assessed using the formalin test (Dubuisson and Dennis, 1977). The rats were placed in open Plexiglas observation chambers for 30 min to allow them to acclimate to their surroundings; then they were removed for formalin administration. Fifty μl of diluted formalin (0.5% for diabetic rats or 1% for non-diabetic rats) were injected subcutaneously into the dorsal surface (Capone and Aloisi, 2004) of the right hind paw with a 30-gauge 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 number of flinches of the injected paw during 1-min periods every 5 min, up to 60 min after injection (Wheeler-Aceto and Cowan, 1991). Flinching was readily discriminated and was characterized as rapid and brief withdrawal, or as flexing of the injected paw. Formalin-induced flinching behavior was biphasic (Dubuisson and Dennis, 1977). The initial acute phase (0–10 min) was followed by a relatively short quiescent period, which was then followed by a prolonged tonic response (15–60 min). At the end of the experiment the rats were sacrificed in a CO_2 chamber.

2.4. Evaluation of antiallodynic activity

Rats were prepared according to the method of Kim and Chung (1992). Animals (weight range, 120–140 g) were anesthetized with a mixture of ketamine/xylazine (45–12 mg/kg, i.p.). After surgical preparation and exposure of the dorsal vertebral column, the L5 and L6 spinal nerves were exposed and tightly ligated with 6-0 silk suture distal to the dorsal root ganglion. For sham operated rats, the nerves were exposed but not ligated. The incisions were closed, and the animals were allowed to recover for 12 days. Rats exhibiting motor deficiency (such as paw-dragging) were discarded from testing.

Tactile allodynia was determined, in spinal nerve ligated rats and diabetic rats of 4 to 6 weeks after injection of streptozotocin, by measuring paw withdrawal in response to probing with a series of calibrated fine filaments (von Frey filaments). The strength of the von Frey stimuli range from 0.4 to 15 g. Withdrawal thresholds were determined by increasing and decreasing stimulus strength eliciting paw withdrawal (Chaplan et al., 1994). The stimulus intensity required to produce a response in 50% of the applications for each animal was defined as “50% withdrawal threshold”. All rats were verified to be allodynic (responding to a stimulus of less than 4 g). Rats not demonstrating allodynia were not further studied.

2.5. Drugs

Benfotiamine was a gift of Merck SA de CV (Mexico City). Benfotiamine was dissolved in carboxymethylcellulose 1% and given orally at a volume ratio of 4 ml/kg. Streptozotocin was dissolved in distilled water.

2.6. Study design

For the study of hyperalgesia, rats received the oral administration of vehicle (carboxymethylcellulose 1%) or benfotiamine (300 mg/kg, p.o.) at different times (10 min, 1 and 2.5 h) before formalin injection (50 μl). Since we observed the best antinociceptive effect at the 2.5 h pretreatment, dose–response curve for benfotiamine was carried out

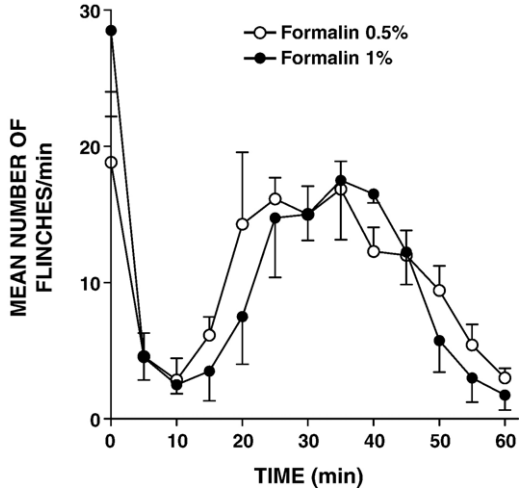

Fig. 1. Time course of the nociceptive behavior induced by subcutaneous injection of formalin to non-diabetic (black circles) and diabetic (white circles) rats. Data are expressed as the mean number of flinches. Data are the means± S.E.M. of 6 animals.

giving vehicle (carboxymethylcellulose 1%) or increasing doses of benfotiamine (10–300 mg/kg) 2.5 h before formalin injection into the right paw. The formalin concentration used in non-diabetic rats was 1%, while 0.5% was used in diabetic rats (2 weeks).

For the study of allodynia, rats received the oral administration of vehicle (carboxymethylcellulose 1%) or increasing doses of benfotiamine (75–300 mg/kg, p.o.) and withdrawal threshold in both spinal nerve-injured and diabetic rats (4–6 weeks) was measured for the next 5 h. Observer was unaware of the treatment in each animal. Rats in all groups were observed regarding behavioral or motor function changes induced by the treatments. This was assessed, but not quantified, by testing the animals' ability to stand and walk in a normal posture, as proposed elsewhere (Chen and Pan, 2001).

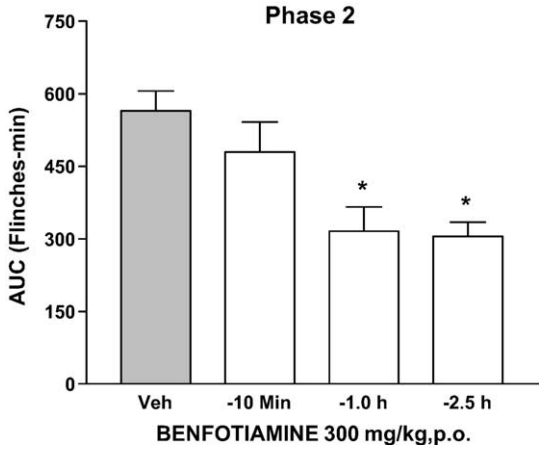

Fig. 2. Effect of benfotiamine on the second phase of the formalin test. Rats received oral administration of benfotiamine at 10 min, 1 and 2.5 h and then an injection of 1% formalin (50 μl) at time zero. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the means± S.E.M. of 6 animals. *Significantly different from the vehicle (Veh) group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

2.7. Data analysis and statistics

All results are presented as means±S.E.M. for 6 animals per group. For the formalin test, curves were made for mean number of flinches against time. The area under the number of flinches against time curves (AUC) for both phases was calculated according to trapezoidal rule. For allodynia, curves were constructed plotting the 50% threshold for paw withdrawal as a function of time. An increase of 50% threshold withdrawal was considered as an antiallodynic effect. Effect was also expressed as the area under the 50% threshold withdrawal against time curve (AUC).

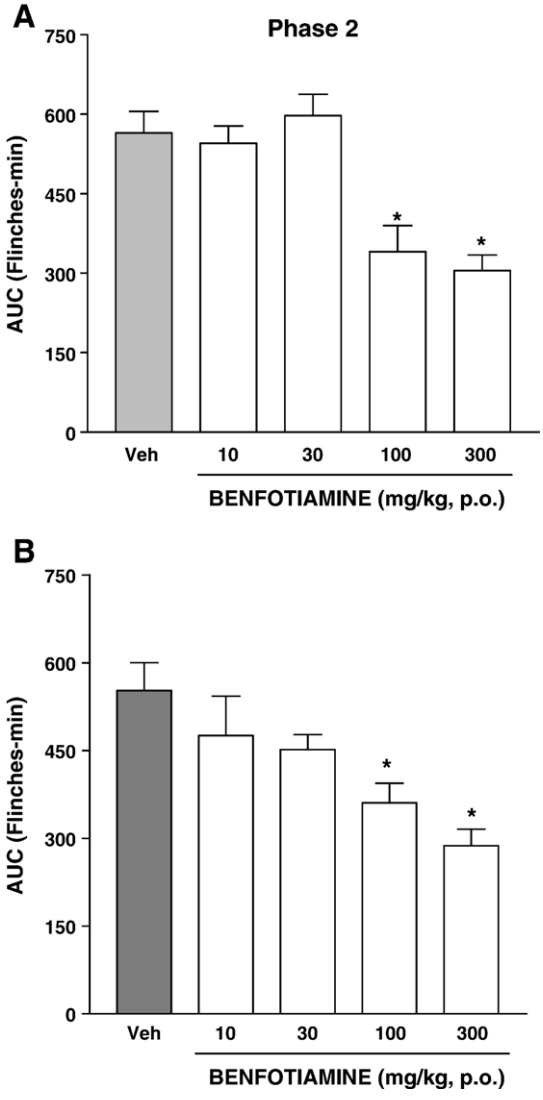

Fig. 3. Antinociceptive effect produced by oral administration of benfotiamine during phase 2 of the formalin test in non-diabetic (A) and diabetic (B) rats. Non-diabetic and diabetic rats received oral administration of benfotiamine (−2.5 h) and an injection of either 1% or 0.5% formalin (50 μl) at time zero, respectively. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the means±S.E.M. of 6 animals. *Significantly different from the vehicle (Veh) group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

Analysis of variance followed by Tukey's test was used to test the significance of differences between treatments. A $P<0.05$ was considered significant.

3. Results

3.1. Antinociceptive effect of benfotiamine in non-diabetic and diabetic rats

Subcutaneous formalin injection into the right hind paw of non-diabetic and diabetic (2 weeks) rats produced a typical pattern of flinching behavior characterized by a biphasic time course (Fig. 1). Phase 1 of the nociceptive response began immediately after formalin administration and then declined gradually in approximately 10 min. Phase 2 began about 15 min after formalin administration and lasted about 1 h (Dubuisson and Dennis, 1977; Porro and Cavazzuti, 1993). Diabetic rats injected with 0.5% formalin (Fig. 1, black circles) displayed a flinching behavior similar to that observed in non-diabetic rats injected with 1% formalin (Fig. 1, white circles), thus suggesting a hyperalgesic effect of diabetes.

Oral administration of benfotiamine (300 mg/kg) significantly reduced formalin-induced nociceptive behavior at 1 and 2.5 h, but not at 10 min, pretreatment (Fig. 2). Therefore, 2.5 h pretreatment time was used in the following experiments.

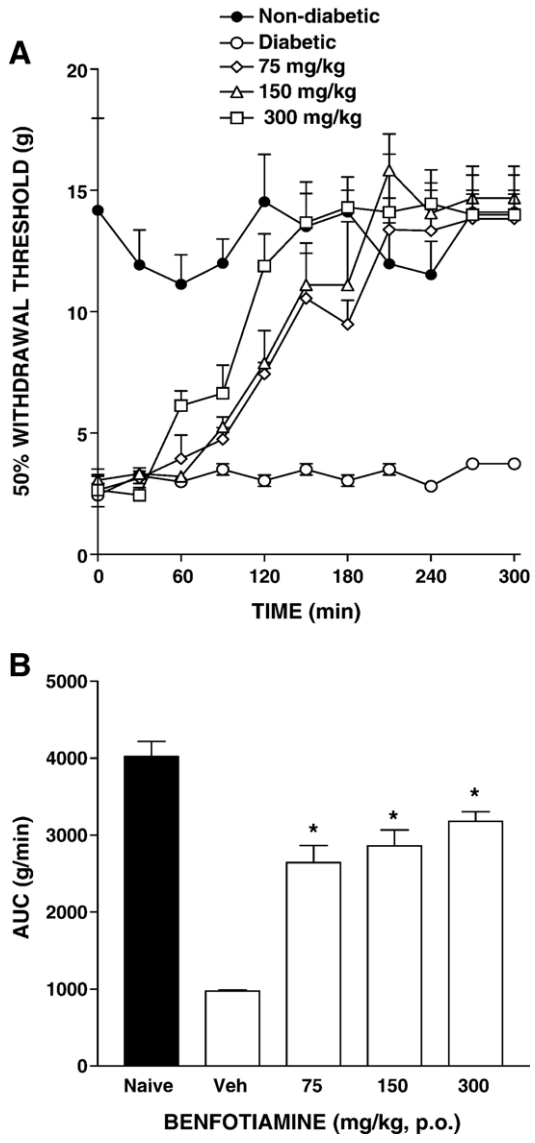

Fig. 4. (A) Time course of the antiallodynic effect of benfotiamine in streptozotocin-pretreated diabetic rats. After diabetes induction, animals were allowed to develop tactile allodynia for 4 to 6 weeks. Animals were treated with oral benfotiamine and withdrawal threshold was measured for the next 5 h. (B) Antiallodynic effect produced by oral administration of benfotiamine in diabetic (4–6 weeks) rats. Data are expressed as the 50% threshold withdrawal against time curve (AUC). *Significantly different from the vehicle (Veh) group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test. In both plots data are the mean±S.E.M. for 6 animals.

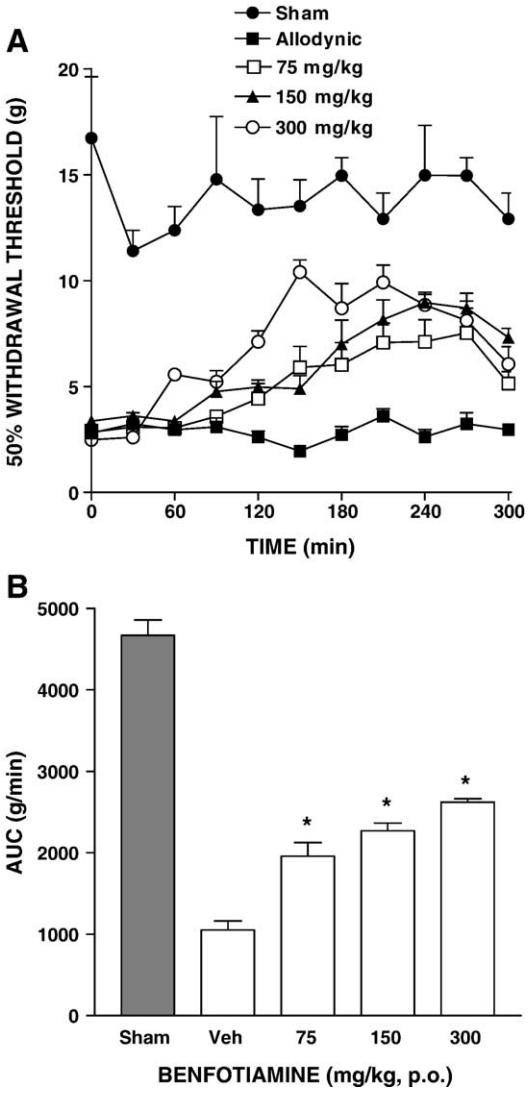

Fig. 5. (A) Time course of the antiallodynic effect of benfotiamine in spinal nerve injury rats. After ligation of L5/L6 spinal nerves, animals were allowed to develop tactile allodynia for 12 days. Then animals were treated with oral benfotiamine and withdrawal threshold was measured for the next 5 h. (B) Antiallodynic effect produced by oral administration of benfotiamine in spinal nerve ligated rats. Data are expressed as the 50% threshold withdrawal against time curve (AUC). *Significantly different from the vehicle (Veh) group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test. In both plots data are the mean±S.E.M. for 6 animals.

Benfotiamine pretreatment significantly reduced ($P<0.05$) flinching behavior in both non-diabetic (Fig. 3A) and diabetic (2 weeks, Fig. 3B) rats.

3.2. Antiallodynic effect of benfotiamine in diabetic and spinal nerve ligated rats

One week after administration of streptozotocin glucose levels increased to about 500 mg/dl and remained high after 6 weeks (data not shown). The weight of the diabetic rats became significantly lower than that of controls by the first week and did not change significantly up to week 6. No autotomy behavior was ever observed during the experiment. Four to six weeks after diabetes induction, a clear tactile allodynia was observed in the streptozotocin-injected rats (Fig. 4A, white circles) compared to the distilled water-injected rats (Fig. 4A, black circles). Oral administration of benfotiamine, but not vehicle (carboxymethylcellulose 1%), increased the withdrawal threshold in diabetic rats (Fig. 4A). Onset for the antiallodynic effect of benfotiamine was between 1 and 2 h and the maximal antiallodynic effect was reached at 2.5 h with the greatest dose tested (300 mg/kg). Benfotiamine significantly reduced streptozotocin-induced tactile allodynia ($P<0.05$) at the 3 tested doses (Fig. 4B). No change in the reflexes was observed in either group, control or treated (data not shown).

On the other hand, ligation of L5 and L6 spinal nerves produced a clear-cut allodynia in rats submitted to the surgery (Fig. 5A, black squares) compared to the sham operated rats (Fig. 5A, black circles). Ligation of spinal nerves did not modify weight gain in these rats compared to the sham operated rats (data not shown). Onset for the antiallodynic effect of benfotiamine in spinal nerve ligated rats was between 1 and 2 h and the maximal antiallodynic effect was reached at about 2.5 h with the greatest dose tested (Fig. 5A). In contrast to diabetic rats, benfotiamine-induced antiallodynic effect tended to disappear at 5 h. Oral administration of benfotiamine, but not vehicle (carboxymethylcellulose 1%), significantly reduced spinal nerve ligation-induced tactile allodynia ($P<0.05$) at the 3 tested doses (Fig. 5B). No change in the reflexes was observed in either group, control or treated (data not shown).

4. Discussion

In the present study we have observed that oral administration of benfotiamine was able to reduce formalin-induced nociception in non-diabetic rats. Moreover, benfotiamine also reduced flinching behavior in diabetic rats (2 weeks). To our knowledge, this is the first report about the antinociceptive and anti-hyperalgesic effect of benfotiamine in a preclinical model of pain in non-diabetic and diabetic rats, respectively. Recent data have demonstrated that thiamine significantly reduces nociception in the *p*-benzoquinone- and acetic acid-induced constriction model and formalin test in mice (Abacioğlu et al., 2000a,b; França et al., 2001). Since benfotiamine is an analogue of thiamine, our results confirm the antinociceptive effect of vitamin B_1 in this kind of pain.

Our results also indicate that benfotiamine is able to reduce tactile allodynia induced either by L5/L6 spinal nerve ligation or administration of streptozotocin in the rat. To the best of our knowledge, this is the first report about the antiallodynic effect of benfotiamine in two well accepted models of neuropathic pain in the rat. Our data agree, however, with previous observations showing that thiamine is able to reduce thermal hyperalgesia in spinal ganglia compression or loose ligation of the sciatic nerve models of neuropathic pain in rats (Wang et al., 2005). Previous open and double-blind clinical trials have shown that benfotiamine is able to significantly reduce neuropathic pain produced by alcoholism (Woelk et al., 1998) or diabetes (Winkler et al., 1999; Haupt et al., 2005). Therefore, our data in rats confirm clinical evidence about the antiallodynic efficacy of benfotiamine.

Diabetes leads to several complications including renal failure, stroke, cardiovascular disease and nerve damage (Obrenovich and Monnier, 2003). It has been hypothesized that diabetes-induced high plasma glucose concentrations are responsible for increased mitochondrial free radical production and subsequent inactivation of glyceraldehyde phosphate dehydrogenase in several cells implicated in these conditions. As a result of the reduced ability of glyceraldehyde phosphate dehydrogenase to process upstream metabolites, three pathways of metabolic damage are activated, namely the advanced glycation end-product formation pathway, the diacylglycerol–protein kinase C pathway, and the hexosamine pathway (Brownlee, 2001). A recent study has reported that benfotiamine blocks the three pathways via activating the pentose phosphate pathway enzyme transketolase. This blockade correlates with the prevention of experimental diabetic retinopathy (Hammes et al., 2003) and nephropathy (Babaei-Jadidi et al., 2003). In addition, previous studies have shown that benfotiamine is able to inhibit the advanced glycation end-product formation pathway and to completely prevent diabetes-induced glycoxidation products in peripheral nerves of diabetic rats (Stracke et al., 2001; Karachalias et al., 2003; Hammes et al., 2003). All these effects could be the basis to explain the anti-hyperalgesic and antiallodynic effect of benfotiamine in diabetic rats.

On the other hand, it has been shown that spinal and local peripheral administration of protein kinase C inhibitors produces antiallodynic and antinociceptive effect in rats previously submitted to spinal nerve ligation (Hua et al., 1999; Yajima et al., 2003) or formalin injection (Aley et al., 2000; Souza et al., 2002), respectively. Since benfotiamine is able to inhibit the diacylglycerol–protein kinase C pathway (Hammes et al., 2003; Babaei-Jadidi et al., 2003), we speculate that the observed antiallodynic, anti-hyperalgesic and antinociceptive effect of this drug may be due to the inhibition of this pathway. However, so far the mechanism of antiallodynic or antinociceptive action of benfotiamine in spinal nerve ligated rats and in the formalin test, respectively, warrants further investigation.

In summary, this study has shown that oral administration of benfotiamine has antinociceptive, anti-hyperalgesic and anti-allodynic activity in rats, suggesting the possible documented clinical use of this drug.

Acknowledgements

The authors greatly appreciate the bibliographic assistance of Héctor Vázquez. Héctor I. Rocha-González and Nadia L. Caram-Salas are CONACYT fellows. This work is part of the B. Sc. dissertation of Gabriela M. Sánchez-Ramírez.

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