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ScienceDirect
European Journal of Pharmacology 577 (2007) 203–210
www.elsevier.com/locate/ejphar

Melatonin reduces formalin-induced nociception and tactile allodynia in diabetic rats

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Received 19 March 2007; received in revised form 17 September 2007; accepted 18 September 2007
Available online 24 September 2007

Abstract

The purpose of this study was to assess the antinociceptive and antiallodynic effect of melatonin as well as its possible mechanism of action in diabetic rats. Streptozotocin (50 mg/kg) injection caused hyperglycemia within 1 week. Formalin-evoked flinching was increased in diabetic rats as compared to non-diabetic rats. Oral administration of melatonin (10–300 mg/kg) dose-dependently reduced flinching behavior in diabetic rats. In addition, K-185 (a melatonin MT_2 receptor antagonist, 0.2–2 mg/kg, s.c.) completely blocked the melatonin-induced antinociception in diabetic rats, whereas that naltrexone (a non-selective opioid receptor antagonist, 1 mg/kg, s.c.) and naltrindole (a selective δ opioid receptor antagonist, 0.5 mg/kg, s.c.), but not 5′-guanidinonaltrindole (a selective κ opioid receptor antagonist, 1 mg/kg, s.c.), partially reduced the antinociceptive effect of melatonin. Given alone K-185, naltrexone, naltrindole or 5′-guanidinonaltrindole did not modify formalin-induced nociception in diabetic rats. Four to 8 weeks after diabetes induction, tactile allodynia was observed in the streptozotocin-injected rats. On this condition, oral administration of melatonin (75–300 mg/kg) dose-dependently reduced tactile allodynia in diabetic rats. Both antinociceptive and antiallodynic effects were not related to motor changes as melatonin did not modify number of falls in the rotarod test. Results indicate that melatonin is able to reduce formalin-induced nociception and tactile allodynia in streptozotocin-injected rats. In addition, data suggest that melatonin MT_2 and δ opioid receptors may play an important role in these effects.

Keywords: Melatonin; Melatonin MT_2 receptor; Naltrexone; Naltrindole; Diabetes; Tactile allodynia

1. Introduction

Diabetes mellitus is one of the most common chronic medical conditions affecting over 100 million people worldwide, of whom up to 60% may develop diabetic neuropathy (Galer et al., 2000). 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 (Sindrup and Jensen, 1999). However, these drugs often have a limited effect or may cause intolerable side effects. Therefore, other options of treatment are needed.

Melatonin (*N*-acethyl-5-methoxytryptamine) is a hormone synthesized primarily in the mammalian pineal gland and secreted into the bloodstream (Vanecek, 1998). This hormone is involved in several biological functions, including circadian rhythms, sleep and analgesia (Morgan et al., 1994; Vanecek, 1998; von Gall et al., 2002; Simonneaux and Ribelayga, 2003; Zahn et al., 2003). Melatonin has been extensively studied in inflammatory pain models in animals (Cuzzocrea et al., 1997; Raghavendra et al., 2000; Pang et al., 2001; Bilici et al., 2002; El-Shenawy et al., 2002; Ray et al., 2004; Wang et al., 2006).

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doi:10.1016/j.ejphar.2007.09.006

Moreover, there is also evidence about its efficacy in neuropathic pain models (Ulugol et al., 2006; Ambriz-Tututi and Granados-Soto, in press).

Melatonin has shown to reduce several diabetes-induced complications in animals such as oxidative–antioxidative status (Anwar and Meki, 2003; Aksoy, 2003) and the pancreatic (Kanter et al., 2006), renal (Derlacz et al., 2007) and liver (Guven et al., 2006) injury. However, its efficacy in hyperalgesia and tactile allodynia in diabetic rats has not been assessed. It is believed that diabetes-induced hyperglycemia causes neural degeneration *via* the increased oxidative stress (Montilla et al., 1998; Nishida, 2005). Thus, we have hypothesized that melatonin could reduce formalin-induced nociception and tactile allodynia in diabetic animals. Therefore, the purpose of this work was to study the possible antinociceptive and antiallodynic effect of melatonin as well as its mechanism of action in diabetic rats.

2. Materials and methods

2.1. Animals

Experiments were performed on adult female Wistar rats (body weight range, 220–240 g) of 10–12 weeks of age. The animals were obtained from our own breeding facilities and had free access to drinking water, but food was withdrawn 12 h before experiments. Under this condition, we observed that streptozotocin produced a greater % of diabetic rats (90%). Experiments were done in normal light/dark cycle and they 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 experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983) and were approved by our local Ethics Committee.

2.2. Induction of diabetes

Rats were injected with streptozotocin (50 mg/kg, i.p.) (Sigma, St. Louis, MO, USA) to produce experimental diabetes (Courteix et al., 1993). Control animals (age-matched) received distilled water. Diabetes was confirmed 1 week after injection by measurement of tail vein blood glucose levels with the glucose meter Ascensia ELITE (Bayer, Mexico City). Two weeks after streptozotocin injection, glycemia was again determined and only animals with a final blood glucose level $\geq$300 mg/dl were included in the study (90%).

2.3. Assessment of nociception

Nociception in non-diabetic and diabetic (2 weeks) rats was assessed using the 0.5% formalin test (Araiza-Saldaña et al., 2005; Juárez-Rojop et al., 2006). 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%) was injected subcutaneously into the dorsal surface 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 a rapid and brief withdrawal, or as a flexing of the injected paw. Formalin-induced flinching behavior was biphasic (Dubuisson and Dennis, 1977; Araiza-Saldaña et al., 2005). 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). Animals were used only once and at the end of the experiment they were sacrificed in a CO_2 chamber.

2.4. Assessment of allodynia

Tactile allodynia was tested in diabetic rats 4 to 8 weeks after streptozotocin injection as previously described (Chaplan et al., 1994; Sánchez-Ramírez et al., 2006). Briefly, 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) were used to determine the 50% paw withdrawal threshold using the up–down method of Dixon (1980). A series of filaments, starting with one that had a buckling weight 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 that 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) of the final von Frey filament used, κ = the value (from table in Chaplan et al., 1994) for the pattern of positive and/or negative responses, and ∂ = the mean difference (in log units) between stimulus strengths. Behavioral tests were performed immediately before and 60 min after drug administration. Threshold was then assessed every 30 min until 4 h. Allodynia was considered to be present when paw withdrawal thresholds were $<$4 g. Diabetic rats not demonstrating allodynia were not further studied.

2.5. Drugs

Streptozotocin, melatonin (*N*-acethyl-5-methoxytryptamine), K-185 (*N*-butanoyl 2-(5,6,7-trihydro-11-methoxybenzo[3,4] cyclohept[2,1-a]indol-13-yl)ethanamine), naltrexone, naltrindole and 5′-guanidinonaltrindole were obtained from Sigma (St. Louis, MO, USA). Streptozotocin was freshly dissolved in distilled water (obtained from a Milli-Q water system), protected from light and immediately administered. Melatonin was dissolved in carboxymethyl cellulose 1% and given orally by a gastric tube at a volume ratio of 4 ml/kg. K-185 was dissolved in 20% dimethylsulfoxide (DMSO) while that naltrexone, naltrindole and 5′-guanidinonaltrindole were dissolved in 0.9% isotonic saline.