Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Accumulation of sorbitol in the sciatic nerve modulates circadian properties of diabetes-induced neuropathic pain hypersensitivity in a diabetic mouse model

Takahiro Akamine [a], Naoki Kusunose [a], Naoya Matsunaga [a, b], Satoru Koyanagi [a, b], Shigehiro Ohdo [a, *]

[a] Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[b] Department of Glocal Healthcare, Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

ARTICLE INFO

Article history:
Received 18 May 2018
Accepted 31 May 2018
Available online xxx

Keywords:
Diabetes
Neuropathic pain
Sorbitol
Na^+/K^+-ATPase
Circadian rhythm

ABSTRACT

The intensity of pain in diabetic neuropathy varies in a circadian time-dependent manner. It is well known that diabetes has two distinct types, which are differentiated based on the cause of the disease. Previous studies have yet to compare the circadian properties of the pain intensity of diabetic neuropathy between type I and type II diabetes. In this study, we demonstrated that the pain intensity of diabetic peripheral neuropathy in a db/db mouse model of type II diabetes showed a significant diurnal oscillation, but such time-dependent oscillation was not detected in a streptozotocin (STZ)-induced type I diabetic mouse model. The polyol pathway-induced accumulation of sorbitol in peripheral nerve cells suppresses Na^+/K^+-ATPase activity, which is associated with the intensity of pain in diabetic neuropathy. In db/db mice, this accumulation of sorbitol in peripheral nerve cells showed significant diurnal oscillation. In addition, pain intensity and Na^+/K^+-ATPase activity were decreased at the peak time of sorbitol accumulation in these mice. Although STZ-induced diabetic mice also showed sorbitol accumulation and Na^+/K^+-ATPase dysfunction, these measures did not oscillate in a time-dependent manner. These findings reveal differences in the circadian properties of pain hypersensitivity in mouse models of type I and type II diabetes, and also provide ideas for developing novel approaches to the management of diabetic neuropathy.

1. Introduction

A study conducted by the International Diabetes Federation in 2017 revealed that over 400 million people in the world have diabetes and predicted that this number will be approximately 700 million in 2045. The most serious problem patients with diabetes face is complications such as peripheral neuropathy, nephropathy, and retinopathy, known as the “triopathy of diabetes.” Because these complications severely decrease the quality of life (QOL) of patients, their prevention and treatment are an important focus of diabetes therapy. Among the triopathy of diabetes, diabetic peripheral neuropathy is likely to develop first. The main symptoms of diabetic peripheral neuropathy are numbness and/or pain in the extremities and the mainstay treatment for diabetes-induced neuropathic pain is pharmacotherapy. However, patients with diabetic neuropathy is sometimes resistant to analgesic drug treatments [1].

Recent developments in our understanding of circadian biology and the availability of tools to characterize the diseases have furthered our knowledge and shown that a variety of pathological conditions are under the control of the circadian clock. For example, asthmatic episodes often occur from midnight to the early morning as airway potency, bronchial responsiveness, and the release of inflammatory factors are increased at night [2]. Consequently, administration of theophylline in the evening effectively suppresses nocturnal asthmatic symptoms [3]. Revealing the circadian properties of disease symptoms permits more effective treatments. A previous human study also suggested that the pain intensity of diabetic neuropathy exacerbates at night [4], but the mechanism has not yet been elucidated.

There are two types of diabetes, which are distinguished based on their underlying cause. In general, patients with diabetes have a

* Corresponding author.
E-mail address: ohdo@phar.kyushu-u.ac.jp (S. Ohdo).

https://doi.org/10.1016/j.bbrc.2018.05.209
0006-291X/

lack of insulin secretion from pancreatic islets (type I diabetes) or have a decrease in insulin secretion or are unable to use insulin effectively (type II diabetes). Patients with both types of diabetes develop neuropathic pain hypersensitivity. The objective of this study is to characterize the circadian properties of neuropathic pain hypersensitivity in animal models of type I and type II diabetes. Since sorbitol accumulation and decreased Na^+/K^+-ATPase activity in the peripheral nerve cells are implicated in the development of pain hypersensitivity in diabetic neuropathy, these biochemical parameters were assessed in both types of diabetic animals.

2. Materials and methods

2.1. Animals and treatments

Five-week-old male ICR mice were purchased from Charles River Japan Inc. (Kanagawa, Japan). Five-week-old male BKS. Cg-+ $Lepr^{db}$/+$Lepr^{db}$/Jcl (db/db) mice and age/sex-matched BKS. Cg-m+/m+/Jcl (wild-type) mice were purchased from CLEA Japan Inc. (Tokyo, Japan). Animals were housed in a light-controlled room at room temperature (24 ± 1 °C) with a humidity of 60 ± 10%. Food and water were available ad libitum. Under the light/dark cycle, a zeitgeber time (ZT) 0 was designated as lights on and ZT12 as lights off. Epalrestat (Wako) was suspended in propylene glycol supplemented with 2% dimethyl sulfoxide and 8% ethanol. Epalrestat (50 mg/kg/day) was subcutaneously injected at a volume of 0.05 mL/10 g body weight every day. Treatment of the type I diabetes mouse model with epalrestat began the day after streptozotocin (STZ; Wako, Osaka, Japan) administration and continued for 21 days. Similarly, db/db mice were treated with epalrestat from the ages of 7–10 weeks old. Animals were treated in accordance with the guidelines stipulated by the Animal Care and Use Committee of Kyusyu University.

2.2. Preparation of the type-I diabetic neuropathic pain model

The STZ-induced (STZ; Wako, Osaka, Japan) diabetes model was induced in ICR mice with a single administration of STZ (200 mg/kg, i. p.), a pancreatic β cell toxin. Body weight and blood glucose levels were assessed after the STZ treatment. Blood glucose levels were determined using an optical reflectance spectroscopy method. Control mice were injected with an equal volume of saline.

2.3. Assessment of diabetes-induced neuropathic pain

Diabetes-induced neuropathic pain was assessed using von Frey filaments (0.02–2.0 g, Muromachi Kikai Co., Ltd., Tokyo, Japan). Mice were placed in plastic cages with a wire mesh floor and allowed to acclimate for 30min before hindpaw mechanical thresholds were tested. The paw withdrawal threshold (PWT) was assessed by the up-down method [5].

2.4. Measurement of plasma insulin levels

Plasma insulin levels were measured using the Morinaga Ultra Sensitive Mouse/Rat Insulin ELISA Kit (MORINAGA, Kanagawa, Japan) according to the manufacturer's protocol.

2.5. Measurement of sorbitol contents in peripheral nerve cells

Sciatic nerves were homogenized in PBS. The samples were centrifuged at 4000 g for 10 min. Aliquots of the supernatant were deproteinized by centrifugal filter units (Merck Milipore). Filtrates were added to a 0.1 M glycine-NaOH buffer (pH 9.5) containing 0.5 mM nicotinamide adenine dinucleotide (NAD) and 0.1-unit sorbitol dehydrogenase. Samples were taken for a spectrofluorometric measurement of sorbitol 30 min after starting the reaction. The excitation and emission wavelengths were 340 and 460 nm, respectively. The amount of sorbitol in the nerve cells was calculated as the difference in the amount of NADH between samples exposed to sorbitol dehydrogenase and those that were not exposed. The amount of sorbitol in nerve cells was expressed as nmol/mg protein.

2.6. Measurement of Na^+/K^+ -ATPase activity in peripheral nerve cells

Sciatic nerves were homogenized in homogenizing buffer (200 mM sucrose, 20 mM Tris-HCl, 2 mM disodium EDTA; pH 7.5). Twenty microliters of the homogenized samples were incubated with 200 mL of incubation buffer (130 mM NaCl, 20 mM KCl, 5 mM MgCl2, 30 mM histidine, 3 mM ATP, 1 mM ouabain) for 30 min at 37 °C. After incubation, these samples were added to 40 μL of 60% perchloric acid and placed on ice for 10 min. The ice-cold samples were added to 300 μL of 2.5% hexaammonium heptamolybdate tetrahydrate and 450 μL of 25 mg/mL Fiske-Subbarow's reducer and incubated for 20 min. After incubation, the samples were centrifuged at 12,000 g for 10 min. The absorbance of the supernatants at 700 nm was measured. Na^+/K^+-ATPase activity was calculated as the difference in ATPase activity between samples exposed to ouabain and those not exposed. Na^+/K^+-ATPase activity in nerves was expressed as μmol Pi/h/mg protein.

2.7. Statistical analysis

A p-value of 0.05 was the criterion for significance. The difference in significance was analyzed by ANOVA and Fisher's least significant difference test among multiple groups.

3. Results

3.1. Differences in circadian properties of neuropathic pain hypersensitivity between type I and type II diabetic mice

To develop a type-I diabetic neuropathy animal model, ICR mice received a single dose of STZ (200 mg/kg, i. p.). This bacterium-derived toxin is known to ablate pancreatic β-cells and induce insulin deficiency, therefore, STZ-induced hyperglycemic animals are often used as a type I diabetes model [6]. In addition, db/db mice were used as a model of type II diabetes. To characterize the circadian properties of neuropathic pain hypersensitivity of type I and type II diabetes, temporal profiles of pain intensity were investigated by the von Frey test. We previously showed that the PWT of STZ-induced diabetic mice gradually decreased and reached the lowest levels 14 days after the STZ treatment [7]. On day 21, the PWT was decreased at all examined time points and did not show significant diurnal variation (Fig. 1 left panel). Similar reductions in the PWT were also observed in 10-week old db/db mice [7]. The PWT of db/db mice was decreased at all examined time points, but exhibited a significant diurnal oscillation ($p < 0.05$, Fig. 1 right panel). These results revealed that circadian properties of neuropathic pain hypersensitivity were different between mouse models of type I and type II diabetes.

3.2. Circadian physiology and behavior in STZ-induced diabetic mice and db/db mice

To evaluate characteristics of diabetes in STZ-induced diabetic mice and db/db mice, temporal profiles of blood glucose levels and plasma insulin levels were investigated. On day 21 post-STZ

Please cite this article in press as: T. Akamine, et al., Accumulation of sorbitol in the sciatic nerve modulates circadian properties of diabetes-induced neuropathic pain hypersensitivity in a diabetic mouse model, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.209