Neuroscience Letters 635 (2016) 83–89

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Effects of 4-phenyl butyric acid on high glucose-induced alterations in dorsal root ganglion neurons

Dilip Sharma, Jitendra Narain Singh*, Shyam S. Sharma*

Electrophysiology Laboratory, Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), S. A. S. Nagar, Mohali, 160 062, Punjab, India

HIGHLIGHTS

- Pathways involving in diabetic neuropathy are still not fully understood.
- ER stress could be triggered by hyperglycemia in primary sensory neurons.
- Hyperglycemia-induced alteration may be blocked by the 4-phenyl butyric acid.

ARTICLE INFO

Article history:
Received 9 September 2016
Received in revised form 14 October 2016
Accepted 20 October 2016
Available online 21 October 2016

Keywords:
Diabetic neuropathy
Dorsal root ganglion
Hyperglycemia
Pain perception
Sodium channel expression

ABSTRACT

Mechanisms and pathways involving in diabetic neuropathy are still not fully understood but can be unified by the process of overproduction of reactive oxygen species (ROS) such as superoxide, endoplasmic reticulum (ER) stress, downstream intracellular signaling pathways and their modulation. Susceptibility of dorsal root ganglion (DRG) to internal/external hyperglycemic environment stress contributes to the pathogenesis and progression of diabetic neuropathy. ER stress leads to abnormal ion channel function, gene expression, transcriptional regulation, metabolism and protein folding. 4-phenyl butyric acid (4-PBA) is a potent and selective chemical chaperone; which may inhibit ER stress. It may be hypothesized that 4-PBA could attenuate via channels in DRG in diabetic neuropathy. Effects of 4-PBA were determined by applying different parameters of oxidative stress, cell viability, apoptosis assays and channel expression in cultured DRG neurons. Hyperglycemia-induced apoptosis in the DRG neuron was inhibited by 4-PBA. Cell viability of DRG neurons was not altered by 4-PBA. Oxidative stress was significantly blocked by the 4-PBA. Sodium channel expression was not altered by the 4-PBA. Our data provide evidence that the hyperglycemia-induced alteration may be reduced by the 4-PBA without altering the sodium channel expression.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Diabetes is a chronic metabolic disease, which is characterized by hyperglycemia, glycosuria and hyperlipidemia that in long-term increases the probability of developing diabetic complication such as macrovascular and microvascular complications which in turn increases mortality and morbidity [2,13]. Microvascular complications include diabetic cardiomyopathy, nephropathy, retinopathy and neuropathy [9]. Diabetic neuropathy is one of the most common chronic complication of diabetes that develops in about 50% of the population with diabetes [46]. Globally, the number of patients with diabetic neuropathy is rapidly increasing. Diabetes is now considered to be the largest global health emergency of this century as about 415 million adults are suffering with diabetes alone [1]. In China about 109.6 million adults are suffering with diabetes followed by India (about 69.2 million) and USA (29.3 million) [1]. Neuropathy is associated with the degenerative condition and spectrum of structure changes characterized by changes in the peripheral nervous system, progressive loss of peripheral nerve axons leading to skin denervation, loss of myelinated fibers, pain, paranodal demyelination and decrease sensation or complete loss of sensation [11]. Hyperglycemia plays a key role in diabetes when to persisting for the longer time it induces development of neuropathy [8]. The main biological mechanisms/pathways that underlies molecular basis of diabetic neuropathy are yet to be uncovered and understand however diabetic neuropathy can be classified by certain pathophysiology's such as by the process of overproduction of

* Corresponding authors.
E-mail addresses: jnsingh@niper.ac.in (J.N. Singh), sssharma@niper.ac.in, shyamsharma14@yahoo.com (S.S. Sharma).

http://dx.doi.org/10.1016/j.neulet.2016.10.038
0304-3940/© 2016 Elsevier Ireland Ltd. All rights reserved.

superoxide, reactive oxygen species (ROS), downstream intracellular signaling pathways and their modulators [17]. Susceptibility of neuronal cells *i.e.* dorsal root ganglion (DRG) to internal or external stress due to hyperglycemia contributes to pathogenesis and progression of neurodegenerative disorders [14].

A recent study suggests that endoplasmic reticulum (ER) stress may play an important role in pathogenesis and progression of neuropathy [3]. The ER plays a key role in newly synthesized protein processing and folding [4]. Hyperglycemia-induced damage to ER leads to ER stress. Further ER stress may leads abnormal ion channel function, gene expression, transcriptional regulation, metabolism and protein folding [34]. ER stress condition is produced by a variety of adverse stimulation such as hyperglycemia, production and accumulation of ROS and many types of inflammatory factors [6,23]. ER stress can be modulated by chemical molecular chaperones like 4-phenyl butyric acid (PBA) [30]. These molecular chaperones rescue cell damage along with providing cytoprotection; while the mechanism involved in this process are unclear, they may be related to the inhibition of oxidative stress [27,32]. It has been hypothesized that 4-PBA could attenuate sodium channels in DRG with hyperglycemia. In this study, we have elucidated the involvement of ER stress by investigating effects of 4-PBA on oxidative stress.

2. Materials and methods

Studies were performed on 4–6 days old neonatal Sprague – Dawley rats. Experimental protocols were approved by institutional animal ethics committee (IAEC) of National Institute of Pharmaceutical Education and Research (14/32 and 15/01).

2.1. Primary cell culture of DRG neurons

DRG neurons were isolated from rat pups by the method described elsewhere [18–20,45]. Briefly, the dorsal surface of rat pups was dipped in the 70% ethanol for five minutes and then rats were euthanized by anesthesia. The vertebral column was carefully removed and placed in a sterile petri-dish comprising ice-cold Mg^{2+} and Ca^{2+}-free oxygenated Dulbecco's phosphate buffer saline (PBS). The vertebral column was cleaned off from muscle tissue and spinal cord was gently pulled from the vertebrae by lateral incision on either side. Then it was transferred with attached DRG neurons to the sterile Petri dish filled with the oxygenated PBS with glucose. The capsular connective tissue of DRG was removed carefully followed by mincing. After mincing, DRG neurons were transferred to the trypsin solutions and incubated for 30 min at 37 °C; then it was centrifuged for 5 min and then plating medium containing serum was added to the pellet to terminate the enzyme activity. The plating medium contains DMEM F-12 HAM containing 10% fetal bovine serum and 1% antibiotic-antimycotic solution. The solution was centrifuged and the supernatant was removed and re-suspended in the plating medium. The DRG neurons were isolated by gentle trituration with fire-polished Pasteur pipette. The cells were plated in the sterile culture dish pre-coated with poly-L-lysine and incubated at 37 °C plus 95% relative humidity and 5% CO_2 [25,31,45]. After isolation of DRG neurons cells were cultured for 24 h and were divided into three groups *i.e.* control (normal glucose, 5.5 mM), hyperglycemia (high glucose, 30 mM) [45] and hyperglycemia with different concentrations 4-PBA.

2.2. Cell viability assay (MTT assay)

To investigate whether cell death is induced by high glucose, DRG neurons cells were incubated in the presence of normal glucose (5.5 mM) and high glucose (30 mM) conditions for 24 h and cell viability was measured by the MTT assay. MTT assay is a sensitive test for measurement of cell viability or cell proliferation based on the reduction of the tetrazolium dye 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide to an insoluble formazan by mitochondrial enzymes associated with the metabolic activity. MTT assay was performed to determine the effect of 4-PBA treatment on the viability of cultured DRG neuronal cells. DRG cells were trypsinized and seeded in 200 μl media per well in a 96 well plate; allowed to incubate (37 °C, 5% CO_2) overnight. After that cells were incubated with normal glucose, high glucose and high glucose with different concentration of 4-PBA for desired period of time in a 5% CO_2, 37 °C incubator. MTT solution (5 mg/ml in PBS) was added to each well and then further incubated (37 °C, 5%CO_2) for 5–6 h to allow the MTT to be metabolized. Media was removed and cells were re-suspended in the 200 μl of DMSO. Then the plates were agitated on a plate shaker at least 30 min prior to data acquisition. The formazan formed were dissolved in dimethyl sulfoxide (200 μl/well) and absorbance was recorded at 550 nm (formation of formazan) and 630 nm [10,42].

2.3. Oxidative stress assays (Glutathione estimation and Assay of intracellular ROS)

2.3.1. Glutathione estimation

DRG neuron cell culture was used for measuring reduced glutathione (GSH) content. DRG neuron cells were cultured, incubated for desired period and treated with high glucose and 4-PBA. Cells were harvested using trypsin-EDTA to micro-centrifuge tubes [2500 rpm, 5 min, 4 °C] and cell pellets were washed with PBS [2500 rpm, 5 min, 4 °C]. After washing cells were re-suspended in 5% 5-Sulfosalicylic Acid (SSA) solution and kept on ice for 20 min. Centrifugation (14,000 × *g*, 20 min, 4 °C) was done and the supernatant was taken to perform "Enzymatic recycling assay". [Enzymatic recycling assay contain such as reagents are the stock solution of all buffers, DTNB, NADPH, Glutathione, preparation of reaction mixture and all reaction] absorbance was taken at 412 nm using spectrophotometric plate reader. A standard curve was generated using reduced glutathione as standard. Protein estimation was performed according to Lowry method and results were expressed as μM/mg of protein [26,29,39].

2.4. Assay of intracellular ROS

ROS play a key role in cellular pathophysiology. In biological system detection of ROS is difficult for several reasons. Sensitive methods are used for detection of intracellular ROS such as the 2′,7′-dichlorodihydrofluorescein diacetate (H2DCF-DA) dye is mostly used and could react with numerous ROS including hydroxyl radical, peroxynitrite and hydrogen peroxide. The cell images were taken by confocal microscope [12,35,45]. The results were quantified using ImageJ-software (NIH, Bethesda, MD). Images were taken and fluorescence quantification was performed using ImageJ software.

2.5. Apoptosis

Acridine orange/ethidium bromide (AO/EB) staining is used to determine apoptosis in cells that are characterized by apoptotic body formation and nuclear changes in cells. Acridine orange is a vital dye and can stain live and dead cells both. EB is only taken up by cells or stain only cells that have lost cytoplasmic membrane integrity. Uniformly green stain appears in live cells. Early apoptotic cells have a bright green nucleus with chromatin condensation and nuclear fragmentation. Late apoptotic neurons were stained with ethidium bromide appear orange with condensed and often fragmented nuclei. In the experiment, the culture media in

Download English Version:

https://daneshyari.com/en/article/5738603

Download Persian Version:

https://daneshyari.com/article/5738603

Daneshyari.com