Biomedicine & Pharmacotherapy 89 (2017) 468–472

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Original article

Neuroprotective effects of octreotide on diabetic neuropathy in rats

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ARTICLE INFO

Article history:
Received 8 November 2016
Received in revised form 31 January 2017
Accepted 9 February 2017

Keywords:
Diabetes mellitus
Diabetic neuropathy
Octreotide
Electromyography

ABSTRACT

The purpose of the present study is to investigate the possible healing effects of octreotide (OCT) on motor performance, electrophysiological and histopathological findings of diabetic neuropathy in a rat model of diabetes mellitus (DM). To induce diabetes, rats were administered a single dose (60 mg/kg) of streptozotocin (STZ). Diabetic rats were treated either with saline (1 ml/kg/day, n = 7) or OCT (0.1 mg/kg/day, n = 7) for four weeks. Seven rats served as control group and received no treatment. At the end of the study, electromyography (EMG), gross motor function (inclined plate test), general histology and the perineural thickness of sciatic nerve were evaluated. At the end of study, weight loss was significantly lower in OCT treated rats than that of saline treated ones ($p < 0.001$). Electrophysiologically, compound muscle action potential (CMAP) amplitudes of the saline treated DM group were significantly reduced than those of controls ($p < 0.0001$). Also, distal latency and CMAP durations were significantly prolonged in saline treated DM group ($p < 0.05$) compared to control. However, treatment of diabetic rats with OCT significantly counteracted these alterations in EMG. Furthermore, OCT significantly improved the motor performance scores in diabetic rats ($p < 0.05$). Histomorphometric assessment of the sciatic nerve demonstrated a significant reduction in perineural thickness in OCT treated group compared to saline group. In conclusion, OCT possesses beneficial effects against STZ-induced diabetic neuropathy, which promisingly support the use of OCT as a neuroprotective agent in patients with diabetic neuropathy.

1. Introduction

Diabetic neuropathy is the most common complication of diabetes mellitus (DM). More than half of diabetic patients develop peripheral neuropathy with varying severity [1]. There are many factors contributing to the pathogenesis of diabetic neuropathy. The possible factors include endoneural hypoxia or ischemia, increased oxidative stress, reduced *myo*-inositol, over activity of the polyol pathway, deficiency of growth factors, and increased glycosylation end products. Interestingly, although there is no full consensus related to these mechanisms the common point is always long-term increased blood sugar levels [2]. Based on these studies, various therapeutic agents such as aldose reductase inhibitors (ARIs), anti-oxidants, selective PKC inhibitors, and neurotrophic factors have been studied to recover peripheral nerve dysfunction in diabetic animals and patients.

Octreotide (OCT), which is a synthetic analogue of somatostatin, inhibits the release of hormones including growth hormone, thyroid-stimulating hormone, insulin, prolactin and glucagon [3]. Also, OCT has protective effects on the multiple organ damage through the mechanism that is linked to inhibition of inflammatory mediators such as proinflammatory cytokines and peptides [4]. OCT exerts anti-oxidant and anti-proliferative effects through scavenging of free radicals and inhibition of transforming growth factor (TGF-β) [5,6]. Previous studies have suggested the efficacy of OCT on both diabetic and non-diabetic autonomic neuropathies [7–9]. The data from a recent experimental study showed that OCT could ameliorate the severity of ileus in experimental pancreatitis by lessening the damage to inhibitory and excitatory motor innervation [10].

Although OCT has been shown to have beneficial effects on diabetic autonomic neuropathy in previous reports, there is still

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http://dx.doi.org/10.1016/j.biopha.2017.02.027
0753-3322/

limited data regarding its therapeutic effects in diabetic peripheral neuropathy. Therefore, in the present study, we aimed to investigate the neuroprotective and neurorestorative effects of systematically administered OCT on diabetic neuropathy. To achieve this, we assessed compound muscle action potentials (CMAPs), gross motor function and histomorphometric alterations in streptozotocin (STZ)-induced diabetic rats.

2. Materials and methods

2.1. Animals

Twenty-one Sprague Dawley rats aged 8 weeks and weighing 160–180 g were included in the study. Animals were fed ad libitum and housed in pairs in steel cages in a temperature-controlled environment (22 ± 2 °C) with 12-h light/dark cycles. The experimental procedures were approved by the Animal Care and Ethics Committee, and complied with the guidelines for animal research established by the National Institute of Health.

2.2. Experimental protocol

DM was induced by intraperitoneal (i.p.) injection of single dose streptozotocin (STZ) (Sigma-Aldrich, Inc.; Saint Louis, MO, USA) (60 mg/kg in 0.9% NaCl, adjusted to pH 4.0 with 0.2 M sodium citrate) for 14 rats [11,12]. Seven rats served as control group and received no drug (Group 1, n = 7). DM was verified after 24 h by evaluating blood glucose levels drawn from tail tips using glucose oxidase reagent strips (Boehringer- Mannheim, Indianapolis). The rats with 250 mg/dl and higher blood glucose levels were included in this study. Then, 14 diabetic rats were randomly divided into 2 groups and were administered with either 1 ml/kg saline (Group 2, DM + saline, n = 7) or 0.1 mg/kg/day OCT (Sandostatin, Novartis) (Group 3, DM + OCT, n = 7) i.p. for 4 weeks. At the end of the study, EMG and gross motor function (inclined plane test) were evaluated. Then, the animals were euthanized and sciatic nerve was removed for histopathological examination.

2.3. Electrophysiological recordings

EMG was obtained three times from the right sciatic nerve and stimulated supra-maximally (intensity 10 V, duration 0.05 ms, frequency 1 Hz, in the range of 0.5–5000 Hz, 40 kHz/s sampling rate) with a Biopac bipolar subcutaneous needle stimulation electrode (BIOPAC Systems, Inc., Santa Barbara, CA, USA) from the Achilles tendon. Compound muscle action potentials (CMAPs) and changes of latency were recorded by subcutaneous unipolar needle electrodes located in the second or third interosseous muscle. Data were evaluated using Biopac Student Lab Pro version 3.6.7 software (BIOPAC Systems, Inc.), with distal latency, duration, and amplitude of CMAP as the parameters. During the EMG recordings, rectal temperatures of the rats were monitored with a rectal probe (HP Viridia 24-C; Hewlett- Packard Company, Palo Alto, CA, USA) and the temperature of each rat was kept at approximately 36 °C–37 °C by using a heating pad [13].

2.4. Inclined plate test

We evaluated gross motor performance in rats, using a sliding apparatus described by Rivlin and Tator [14], 1 month following diabetes induction with STZ. The sliding apparatus was a 50 cm × 30 cm stainless steel plane. The maximum angle was then determined at the moment just when a limb of the rat slipped in order to maintain body position. The test was performed three times for each head position and averaged. Each trial was performed after a 1-min interval.

2.5. Histopathological examination

Formalin-fixed sciatic nerve sections (4 μm) were stained with hematoxylin and eosine. The thickness of the sciatic perineurium nerve was measured with an Olympus C-5050 digital camera mounted on an Olympus BX51 microscope.

2.6. Statistical analysis

Data analyses were performed using SPSS version 15.0 for Windows. The groups of parametric variables were compared with the analysis of variance (ANOVA) and post hoc Tukey HSD test. Results are given as mean ± standard error of mean (SEM). A value of $p < 0.05$ was accepted as statistically significant.

3. Results

3.1. Evaluation of plasma glucose levels and body weight

All animals were monitored daily for behavior and health conditions throughout the study. As expected, diabetic animals demonstrated typical features of hyperglycemia, including polydipsia, polyuria, and weight loss. The alterations in body weight of rats throughout the study are summarized in Table 1. The comparison of final body weights of the groups revealed significant weight loss in both saline and OCT administered groups compared to control group ($p < 0.0001$ and $p < 0.05$, respectively). Also, a significant difference was observed between saline and OCT administered groups ($p < 0.001$). The plasma glucose levels in both saline and OCT administered groups were significantly increased when compared with control group ($p < 0.0001$); however there was no significant difference between these groups in terms of glucose levels ($p > 0.05$) (Table 1).

3.2. Evaluation of EMG

Fig. 1 represents a sample of the compound muscle action potential (CMAP) recorded from the sciatic nerves of rats. Table 2 demonstrates the alterations in EMG parameters of the study groups. ANOVA results revealed significant differences among the groups ($p < 0.005$ for CMAP amplitude, CMAP duration, and distal latency). CMAP amplitude, which reflects axonal degeneration, was significantly decreased in saline-treated group, when compared with control group ($p < 0.0001$). Additionally, distal latency and CMAP durations were found to be prolonged in saline group compared to control ($p < 0.05$). Treatment of diabetic rats with OCT significantly recovered these alterations, as shown in Table 2 ($p < 0.0001$ for CMAP amplitude, $p < 0.05$ for distal latency and CMAP duration).

3.3. Evaluation of gross motor performance

We determined the effects of OCT on gross motor performance of the rats by inclined plate test. The saline treated diabetic rats

Table 1
Alterations in plasma glucose levels and body weight of the rats.

	Initial weight (g)	Final weight (g)	Glucose (mg/dl)
Control	168.5 ± 10.2	170.6 ± 10.5	85.9 ± 9.2
DM + saline	175.4 ± 16.5	125.4 ± 15.8*	455.8 ± 16.3*
DM + OCT	178.3 ± 12.4	152.9 ± 13.3#	435.8 ± 12.1*

Data are shown as mean ± S.E.M.
* $p < 0.0001$ DM + saline vs. Control.
# $p < 0.001$ DM + OCT vs. DM + saline.