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Research article

Sigma 1 receptor mediated HMGB1 expression in spinal cord is involved in the development of diabetic neuropathic pain

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ABSTRACT

No study has been conducted to examine the interactions of sigma-1 receptor (Sigma-1R) and high mobility group box 1 protein (HMGB1) in the development of diabetic peripheral neuropathy. Thus, we examined the effects of streptozotocin (STZ) treatment on expression of HMGB1 in subcellular levels in the dorsal root ganglion (DRG) in both wild-type and Sigma-1R$^{-/-}$ mice and evaluated the effects of repeated intrathecal administrations of selective Sigma-1R antagonists BD1047, agonist PRE-084, or HMGB1 inhibitor glycyrrhizin on peripheral neuropathy in wild-type mice. We found that STZ-induced tactile allodynia and thermal hyperalgesia was associated with increased total HMGB1 expression in DRG. STZ treatment promoted the distribution of HMGB1 into cytoplasm. Furthermore, STZ induced modest peripheral neuropathy and did not alter HMGB1 levels in DRG or the distribution of either cytoplasmic or nuclear HMGB1 in Sigma-1R$^{-/-}$ mice compared to sham control mice. Additionally, repeated stimulation of Sigma-1R in the spinal cord induced tactile allodynia and thermal hyperalgesia at 1 week. This phenomenon was associated with increased cytoplasmic HMGB1 translocation and HMGB1 expression in DRG. Finally, we found that repeated blockade of either Sigma-1R or HMGB1 in the spinal cord after STZ treatment prevent the development of tactile allodynia and thermal hyperalgesia at 1 week. These effects were associated with decreased cytoplasmic HMGB1 translocation and HMGB1 expression in DRG. Taken together, our results suggest that Sigma-1R-mediated enhancement of HMGB1 expression in the DRG is critical for the development of peripheral neuropathy in type 1 diabetes.

1. Introduction

Diabetic neuropathy is one of the major complications of diabetes and a frequent cause of peripheral neuropathy, affecting approximately 50% of both type 1 and type 2 diabetic patients [7]. However, current treatments can only provide partial pain relief in about 30 percent of patients [14]. Therefore, effective therapeutics for diabetic neuropathy is urgently needed.

It has been shown that inhibition of Sigma-1R induces antinociception [24,33]. Sigma-1R knockout mice exhibited reduced nociceptive responses in capsaicin sensitization [8], in sciatic nerve injury [6], and in the formalin test [4], as well as in chemotherapy-induced cold and mechanical allodynia [18]. Recently, studies have shown that Sigma-1R is involved in the development of neuropathic pain in obesity-associated type 2 diabetes [20,26]. However, it is still not well studied about the molecular mechanisms of Sigma-1R that are involved in the development of diabetic neuropathy, particularly in type 1 diabetes.

High-mobility group Box 1 (HMGB1) is a DNA-binding protein located in the nuclei of most mammalian cells, which plays a critical role in structural and transcriptional activities by binding to chromatin. Emerging evidence has shown that HMGB1 is a proinflammatory mediator of chronic pain development, including neuropathic pain [2]. In an animal model of type 2 diabetes, the development of mechanical allodynia is associated with the upregulation of HMGB1 protein in the spinal cord, and intrathecal injection of the neutralizing antibody against HMGB1 inhibited mechanical allodynia [21]. However, whether Sigma-1R is involved in STZ induced neuropathic pain through modulation of spinal HMGB1 is unclear.

2. Materials and methods

2.1. Animals

The Sigma-1R knockout (Sigma-1R$^{-/-}$) mice were maintained in a temperature- and humidity-controlled vivarium at the Animal Research Center of The Second Hospital of Shandong University under 12:12 h light/dark cycle. All animals were given food and water *ad libitum*. All

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wild-type mice (n = 64) and Sigma-1R$^{-/-}$ mice (n = 16) were used for both behavioral tests and biological assay, as described below. All animal experiments were approved by the Institutional Animal Care and Use Committee of The Second Hospital of Shandong University.

2.2. STZ-Induced diabetic neuropathy in mice

Diabetes was induced in 8-week-old Sigma-1R$^{-/-}$ or wild-type mice by intraperitoneal injection of STZ (200 mg/kg). The onset of the diabetic state was assessed by the presence of hyperglycemia. One week after the STZ injection, mice with a blood glucose level > 16.7 mmol/L were selected to be used for experiments. At the end of the experiments, the blood glucose level in sigma-1R$^{-/-}$ mice was 19.2 ± 1.3 mmol/L, and the blood glucose level in wild-type mice was 18.9 ± 1.7 mmol/L. No difference was observed in the level of blood glucose.

2.3. Intrathecal administration

Immediately after STZ injections, intrathecal administrations of vehicle (i.e., phosphate buffered saline) or BD1047 (selective Sigma-1R antagonist; 30 μM), or glycyrrhizin (HMGB1 inhibitor; 30 μM) were performed once a day for 7 days through the intervertebral space in unanesthetized mice between the L5 and L6 of the spinal cord. Behavioral tests were conducted at about 10 min after the last injections. The doses of BD1047 and glycyrrhizin used in our study were chosen based on literature [9,28]. In separate groups of wild-type mice, intrathecal administrations of vehicle (i.e., phosphate buffered saline) or PRE-084 (selective Sigma-1R agonist; 0.6 μM) were performed once a day for 7 days using the same methods described above.

2.4. Behavioral tests

All behavioral tests were performed in mice one week after STZ injection. Mechanical withdrawal thresholds using von Frey monofilaments were then measured to evaluate peripheral neuropathy. The up-down method was used to determine fifty percent withdrawal thresholds. After von Frey assay, a hot plate test (55 °C) was conducted to measure thermal sensitivity in mice. The latency to lick the front or hind paws was calculated. All the calculations were conducted by examiners who were blinded to the experimental conditions.

2.5. Western blot analysis

Immediately after the behavioral test was completed, animals were sacrificed and transcardially perfused with saline and tissue was removed and frozen immediately with liquid nitrogen and stored at −80 °C. The fresh frozen L4/L5 DRG tissue samples were homogenized in radioimmunoprecipitation assay (RIPA) buffer with protease/phosphatase inhibitors. Bicinchoninic acid BCA protein assay (Thermo Fisher Scientific, Shanghai, China) was used to determine protein concentration. Samples (40 μg/lane) were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane. After incubation in 10% non-fat milk blocking solution overnight at 4 °C, the membrane was incubated with rabbit anti-HMGB1 (Abcam, Shanghai) or rabbit anti-Sigma-1 receptor (Abcam, Shanghai) followed by incubation with horseradish peroxidase-coupled anti-rabbit secondary antibody (Abcam, China). The membrane was re-probed with a monoclonal anti β-actin antibody (Sigma Aldrich, MO, USA). Immunopositive bands were detected by enhanced chemiluminescence (ECL) and measured by a densometric analysis.

2.6. Nuclear and cytoplasmic extraction

NE-PER Nuclear and Cytoplasmic Kits (Thermo Fisher Scientific, Shanghai, China) was used to prepare nuclear and cytoplasmic extracts. Fresh L4/5 DRG tissue were collected and stored at −80 °C. Monoclonal lamin B, nuclear protein, (1: 1000; Santa Cruz Biotechnology, Shanghai, China) and monoclonal α Tubulin, cytoplasmic protein (1:1000; Santa Cruz Biotechnology, Shanghai, China) were used as loading controls.

2.7. Statistical analysis

IBM SPSS Statistics (version 22.0) was used for all statistical analyses of results. All quantitative data were represented as the mean ± SEM. Data were analyzed using one way or mixed-factorial analyses of variance (ANOVAs), where appropriate. Significant ANOVA main and interaction effects were further investigated using Tukey *post hoc* tests, when appropriate. Alpha was set at 0.05.

3. Results

3.1. Effects of STZ treatment on tactile allodynia and thermal hyperalgesia and on the expression of Sigma-1R and HMGB1 in dorsal root ganglion of wild-type mice

All behavioral testing were performed after 1 week on either saline or STZ treatment. STZ-treated mice developed tactile allodynia and thermal hyperalgesia at 1 week, compared to saline-treated mice (*t*-test, $p < .01$; Fig. 1A and B).

Furthermore, we found that there was an increased in the expression of Sigma-1R in the RDGs at 1 week after STZ treatment, compared to saline (*t*-test, $p < .01$; Fig. 1C). We also found that there was an increase in cytoplasmic HMGB1 protein expression compared to sham control (Fig. 1D, n = 8/group, Students *t*-test, $p < .05$) and a decrease in nuclear HMGB1 protein expression compared to sham control (Fig. 1E, n = 8/group, Students *t*-test, $p < .05$). Furthermore, total HMGB1 protein content in the L4/5 DRG was also increased in STZ-treated mice (Fig. 1F, n = 8/group, Students *t*-test, $p < .05$).

3.2. Effects of STZ treatment on tactile allodynia and thermal hyperalgesia and on the expression of Sigma-1R and HMGB1 in dorsal root ganglion of Sigma-1R$^{-/-}$ mice

We then found that Sigma-1R$^{-/-}$ mice exhibited a modest thermal hyperalgesia or tactile allodynia after STZ treatment at 1 week, compared to saline treatment (Fig. 2A and B). We verified that the levels of Sigma-1R expression in the DRG in wild type and Sigma-1R $^{-/-}$ mice (Fig. 2C).

We found that in Sigma-1R$^{-/-}$ mice, STZ treatment did not alter the cytoplasmic HMGB1 protein expression compared to sham control (Fig. 2D) or nuclear HMGB1 protein expression compared to sham control (Fig. 2E). Furthermore, total HMGB1 protein content in the L4/5 DRG was not altered in STZ-treated mice (Fig. 2F).

3.3. Effects of repeated intrathecal administration of BD1047 on STZ-induced tactile allodynia and thermal hyperalgesia and on the expression of HMGB1 in dorsal root ganglion of wild-type mice

Mice receiving intrathecal injections of vehicle (once daily for 7 days) showed tactile allodynia and thermal hyperalgesia after STZ treatment at 1 week (Fig. 3A and B). However, repeated intrathecal injections of BD1047 (30 μM) increased paw withdrawal threshold in wild type mice, compared to vehicle (Fig. 3A). Similarly, repeated intrathecal injections of BD1047 (30 μM) increased paw licking latency in wild type mice, compared to vehicle (Fig. 3B).

Furthermore, we found that in STZ-treated mice, BD1047 intrathecal injections reduced the cytoplasmic HMGB1 protein expression compared to vehicle control (Fig. 3C) but did not alter nuclear HMGB1 protein expression compared to vehicle control (Fig. 3D). Finally, total HMGB1 protein content in the L4/5 DRG was reduced by BD1047 injections in STZ-treated mice (Fig. 3E).