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Research report

Administrations of thalidomide into the rostral ventromedial medulla alleviates painful diabetic neuropathy in Zucker diabetic fatty rats

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ABSTRACT

The rostral ventromedial medulla (RVM) plays a critical role in pain signal transmissions. However, the mechanisms of RVM in type 2 diabetic neuropathy are still poorly understood. Therefore, we evaluated the mechanisms within the RVM in the modulation of neuropathic pain in type 2 diabetes. To this end, we used Zucker diabetic fatty (ZDF) rats to examine the levels of TNFα, IL-1β, and NF-κB in the RVM during the development of neuropathic pain in type 2 diabetes, and evaluated the effects of intra-RVM microinjections of thalidomide on the levels of TNFα, IL-1β, and NF-κB in the RVM and mechanical allodynia and thermal hyperalgesia induced by type 2 diabetes. We found that ZDF rats became hyperglycemic and exhibited increased levels of TNFα, IL-1β, and NFκB in the RVM at the age of 13 weeks. Intra-RVM administrations of thalidomide dose-dependently attenuated mechanical allodynia and thermal hyperalgesia, and this phenomenon was associated with reduced levels of TNFα, IL-1β, and NFκB in the RVM, without altering serum levels of TNFα or IL-1β. These results suggested that supraspinal mechanisms of thalidomide play a critical role in modulations of type 2 diabetes induced neuropathic pain, which is likely mediated by TNFα and IL-1β in the RVM.

1. Introduction

Diabetic neuropathy is the most common complication of diabetes mellitus. It can affect peripheral sensory neurons, and occur in a large percent of adult diabetic patients (Boulton et al., 2005). Excessive sensitivity to nociceptive stimuli or perceiving normal stimuli as painful stimuli are often experienced in patients with diabetic neuropathy (Maritim et al., 2003; Obrosova, 2009; Yasuda et al., 2003), which results in a significant adverse effect on quality of life measures (Schmader, 2002). While it remains largely unclear about the etiology of pain in diabetic neuropathy, more and more evidence suggests that the activation of inflammatory cascades in the peripheral and central nervous system may play a role in the development and persistence of neuropathic pain states induced by diabetes (Selvarajah et al., 2011; Sytze Van Dam et al., 2013; Vincent et al., 2013).

Studies have shown that systemic immune activation occurs in diabetes patients with painful neuropathy, including increased interleukin (IL)-2 and tumor necrosis factor (TNF)α mRNA and protein levels in blood (Uceyler et al., 2007). Additionally, studies have shown that serum TNFα level is enhanced in type 1 diabetes patients (Doupis et al., 2009; Gonzalez-Clemente et al., 2005). Furthermore, a recent study has demonstrated that there is an elevation of a significant number of pro-inflammatory cytokines such as TNFα and interleukin-1 in the dorsal root ganglion (DRG) in a rat model of type 2 diabetes. This study suggested that pro-inflammatory cytokines in the nervous system may play a critical role in neuropathic pain of type 2 diabetes. However, it is still unknown the neural substrates that might be involved in modulations of neuropathic pain of type 2 diabetes.

As an important part of the descending nociceptive system, the rostral ventromedial medulla (RVM) projects to the spinal cord and trigeminal brain stem nuclei and plays a critical role in control of pain signal transmissions (Millan, 2002; Ossipov et al., 2010; Ren and Dubner, 2002). It has been shown that long lasting activation of descending modulatory neurocircuits is closely related to hyperalgesia and allodynia in animal models of persistent pain (Dubner and Ren, 2004; Gebhart, 2004; Porreca et al., 2002; Vanegas and

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Schaible, 2004). Furthermore, peripheral inflammation (Guo et al., 2006; Urban and Gebhart, 1999; Wei et al., 1999) and nerve injury (Kovelowski et al., 2000; Pertovaara et al., 1996) may activate the descending modulatory neurocircuits and lead to the development of persistent pain. Importantly, recent studies have shown that prolonged elevations of cytokines TNFα and IL-1β in the RVM after chronic constriction injury of the rat infraorbital nerve play a critical role in maintaining persist pain state (Wei et al., 2008). Despite that numerous studies have illustrated that role of RVM in descending pain modulation, the mechanisms of RVM in the involvement of diabetic neuropathy, particular in type 2 diabetes neuropathy, are still poorly understood.

Previous studies have demonstrated that thalidomide can prevent thermal hyperalgesia and allodynia in an animal model of neuropathic pain (Cata et al., 2008). Furthermore, recent studies have shown that thalidomide can strongly prevent the mechanical allodynia and thermal hyperalgesia in streptozotocin (STZ)-induced type 1 diabetic neuropathy (Chauhan et al., 2012; Huang et al., 2014; Taliyan and Sharma, 2012). It has been reported that thalidomide can reduce the synthesis of TNFα and other pro-inflammatory cytokines including IL-1β (Sampaio et al., 1991; Singhal and Mehta, 2002). Given that TNFα and IL-1β are two important cytokines to induce NF-κB activity (Hasko et al., 1996), these findings suggested that activation of NF-κB plays an important role in development of neuropathic pain in type 1 diabetes. In fact, inhibition of NF-κB in DRG neurons following peripheral nerve injury attenuates neuropathic pain (Fu et al., 2010; Ma and Bisby, 1998; Meunier et al., 2007; Niederberger et al., 2007; Sun et al., 2006; Tegeder et al., 2004; Zang et al., 2010). However, the role of TNFα, IL-1β, and NF-κB in the RVM in modulation of neuropathic pain in type 2 diabetes remains unknown.

Therefore, this study was designed to evaluate the neuropharmacological mechanisms within the RVM in the modulation of neuropathic pain in type 2 diabetes. To this end, we used Zucker diabetic fatty (ZDF) rats to examine the levels of TNFα, IL-1β, and NF-κB in the RVM during the development of neuropathic pain in type 2 diabetes. We then evaluated the effects of intra-RVM injections of thalidomide on the levels of TNFα, IL-1β, and NF-κB in the RVM and mechanical allodynia and thermal hyperalgesia that were associated with type 2 diabetes.

2. Materials and methods

2.1. Animals

Male Zucker diabetic fatty (ZDF; fa/fa) rats and control (Lean; fa/+) rats at the age of 7 weeks were purchased from Charles River Laboratories (Beijing, China), and were acclimated in the Animal Center of The First Hospital of Shijiazhuang for one week before subsequent experiments. Rats were housed in separated cages in a room with a 12:12 light cycle, and were given food (Purina 5008 rat chow, International Product Supplies Ltd., Shanghai, China) and water ad libitum. All animals were maintained under a 12:12 h cyclic lighting schedule with 21.0 °C–23.0 °C and 50%–60% humidity. All animal experiments were approved by the Institutional Animal Care and Use Committee of The First Hospital of Shijiazhuang. The housing and treatment of the rats followed the guidelines of the "Guide for the Care and Use of Laboratory Rats" (Institute of Laboratory Animal Resources, Commission on Life Sciences 2011).

2.2. Blood glucose and weight monitor

Starting from the ninth week of rat age, weight was measured daily and blood glucose measurements (glucose diagnostic reagents; Sigma, St. Louis, MO, USA) were recorded every week. Rats were fasted for 3 h prior to collection of blood collection from the tail. The onset of diabetic conditions was defined as blood glucose levels higher than 13.3 mmol/L. Consistent with literature, the animals did not develop significant ketoacidosis or prostration during this time period (Chen and Levine, 2003; Hong et al., 2004).

2.3. Stereotaxic surgery

One week after rats arrived in the facility, animals were anesthetized with 2–3% isoflurane in a gas mixture of 30% O_2 balanced with nitrogen, and placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA). A midline incision was made after infiltration of Bupivacaine (0.25%) into the skin. The skull was exposed and a guide cannula (26 gauge, Plastics One, Roanoke, VA, USA) was implanted at the RVM using the following stereotaxic coordinates (A/P 10.8 mm, M/L ±0.20 mm, and D/V −7.0 mm) (Paxinos and Watson, 2005). Stainless steel screws and dental cement (Durelon; Thompson Dental Supply, Raleigh, NC, USA) were then used to secure the cannula onto the skull. A dummy cannula (Plastics One, Roanoke, VA, USA) was inserted to prevent contamination and maintain patency of the guide cannula. Rats recovered on a heating pad for up to 2 h post-procedure and were then singly housed while recovering for 7 days.

2.4. Intra-RVM microinjections

Rats were adapted to the microinfusion procedure prior to intra-RVM injections of thalidomide. To this end, rats were mildly restrained by experimenter in hands, and an injection cannula was inserted into the guide cannula to a depth 2 mm below the tip of the guide cannula. The injector was left in place for 4 min, but no fluid was infused. Intra-RVM microinjections were started when rats reach the age of 13 weeks, since our pilot study and other studies have shown that ZDF rats became diabetic after 13 weeks old (Chen et al., 2015). Microinjections were made directly into the RVM through a 30-guage stainless steel injector. Rat received intra-RVM microinjections of saline (0.5 μl/site; n = 10) or one dose of thalidomide (2.5 or 5 μg/0.5 μl/site; n = 10/dose). The infusion rate was 0.25 μl/min. After the microinjection, rats were handled by experimenter for 5 min before being placed back to home cage. Microinjections were conducted daily for 7 consecutive days.

2.5. Behavioral analysis

All behavioral testing were conducted between 1:00 PM and 3:00 PM on Friday of each week from week 9 to week 16 for 8 consecutive weeks. Prior to the first behavioral testing, rats were acclimated to the behavioral apparatus and equipment for a minimum of 2 days. On test days, rats were placed in the behavioral apparatus and allowed to acclimate to the environment for 30 min. To test rat sensitivity to a mechanical stimulus, Von Frey assay was conducted. Rats were placed in a clear plastic cage on top of a wire mesh grid that allowed access to their hind paws for the duration of the analysis. Using the up-down method to determine fifty percent withdrawal thresholds (Carter and Shieh, 2010; Kruger, 2001), mechanical withdrawal thresholds using von Frey monofilaments were measured to examine the effects of daily intra-RVM microinjections of thalidomide on diabetic neuropathy. A hot plate test was also performed to examine the effects of daily intra-RVM microinjections of thalidomide on thermal sensitivity. The mice were placed on a hot plate maintained at 55 °C, and the latency to lick the front or hind paws was monitored with a video camera and recorded on videotape. Mechanical withdrawal thresholds and the latency time were then analyzed by blind examiners.