European Journal of Pharmacology 680 (2012) 28–33

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

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

Neuropharmacology and Analgesia

The effects of galanin on neuropathic pain in streptozotocin-induced diabetic rats

Xiaofeng Xu [a], Zhen Liu [a], Huaxiang Liu [b], Xiangdong Yang [c], Zhenzhong Li [a,*]

[a] Department of Anatomy, Shandong University School of Medicine, Jinan 250012, China
[b] Department of Rheumatology, Shandong University Qilu Hospital, Jinan 250012, China
[c] Department of Nephrology, Shandong University Qilu Hospital, Jinan 250012, China

ARTICLE INFO

Article history:
Received 28 August 2011
Received in revised form 7 January 2012
Accepted 13 January 2012
Available online 26 January 2012

Keywords:
Diabetes
Neuropathic pain
Galanin
Galanin receptor
Dorsal root ganglion
Spinal dorsal horn

ABSTRACT

Diabetic neuropathy is a common complication associated with diabetes and is frequently painful. However, mechanisms responsible for diabetic neuropathic pain are still unclear. Experimental evidence has shown that the galanin and its receptor are involved in pain sensitization. The objective of the present study was to investigate the role of galanin and its receptor antagonist or agonist on neuropathic pain in streptozotocin-induced diabetic rats. The expression of galanin, galanin receptors 1 and 2 in dorsal root ganglion (DRG) and spinal dorsal horn (SDH) in diabetic rats were detected by Western blot assay. The effects of galanin, galanin receptor antagonist M35, galanin receptor 1 agonist M617, and galanin receptor 2 agonist AR-M1896 on neuropathic pain were evaluated by mechanical stimuli. The results showed that (1) the diabetic rats showed a significant mechanical hyperalgesia between 4 and 12 weeks; (2) galanin receptor 1 expression decreased in SDH in diabetic rats; (3) galanin receptor 2 expression decreased in DRG and SDH in diabetic rats; (4) intrathecal administration of exogenous galanin attenuated diabetic neuropathic pain, this effect could be blocked by pre-treatment with galanin receptor antagonist M35; and (5) intrathecal administration of galanin receptor 1 agonist M617, but not galanin receptor 2 agonist AR-M1896, attenuated diabetic neuropathic pain. These results imply that galanin acts through receptor 1, but not galanin receptor 2, to exert analgesic effect in diabetic neuropathic pain and is one of the potential therapeutic targets on diabetic neuropathic pain sensitization.

1. Introduction

Neuropathic pain of diabetic patients is a serious problem worldwide and lack of effective treatment currently and mechanisms responsible for diabetic neuropathic pain are still unclear. Peripheral sensory diabetic neuropathy is characterized by morphological, electrophysiological and neurochemical changes to a subpopulation of primary afferent neurons (Zaruba et al., 2007). Dorsal root ganglion (DRG) is involved in diabetic neuropathy (Chattopadhyay et al., 2009; Zheng et al., 2010). DRG has been identified as the target tissue in diabetic somatosensory neuropathy. In peripheral sensory diabetic neuropathy, abnormalities were not only in peripheral nerve fibers but also in the DRG primary sensory neuronal cell bodies (Shimoshige et al., 2009). Spinal dorsal horn (SDH) is involved in nociceptive transmission in painful diabetic neuropathy (Li et al., 2010). Diabetic neuropathic pain is associated with increased glutamatergic input in the SDH (Li et al., 2010). The expression of a nociceptive activation marker Fos protein increased at SDH in diabetic neuropathy rats (Morgado et al., 2010). Spinal cord stimulation offers an effective and safe therapy for chronic diabetic neuropathic pain (de Vos et al., 2009). The underlying pathophysiological mechanisms associated to hyperexcitability and spontaneous hyperactivity of spinal cord neurons in painful diabetic neuropathy are not clear (Morgado et al., 2008).

Neurotrophic peptides have been shown to be effective in preventing or reversing diabetic neuropathy (Chattopadhyay et al., 2009). Galanin, a 29-amino-acid neuropeptide (30-amino-acid in human), is widely distributed throughout the nervous system and is involved in the regulation of manifold functions including nociception, developmental and trophic effects (Brumovsky et al., 2006a; Shi et al., 2006; Yang et al., 2008). Galanin also participates in energy homeostasis and glucoregulation (Jiang et al., 2009; Zorrilla et al., 2007). Galanin expression changes in DRG neurons in acute and chronic inflammation pain models (Landry et al., 2005) and in high glucose treated DRG in vitro (Xu et al., 2012). Galanin in primary sensory neurons may confer analgesia following injury (Kennedy and Zochodne, 2004). Galanin may play a role in processing of sensory information, especially pain, through galanin receptors 1 and 2 both in DRG and spinal cord level (Brumovsky et al., 2006a; Landry et al., 2003, 2005; Shi et al., 2006). It affects pain threshold and has developmental and trophic effects (Brumovsky et al., 2006a; Jimenez-

* Corresponding author at: Department of Anatomy, Shandong University School of Medicine, 44 Wenhua Xi Road, Jinan, Shandong Province 250012, China. Tel.: +86 158 6379 3602; fax: +86 531 8603 3058.
E-mail addresses: xxf820929@yahoo.com.cn (X. Xu), zhen@sdu.edu.cn (Z. Liu), huaxiangliu@yahoo.com.cn (H. Liu), yangxiangdong@medmail.com.cn (X. Yang), zli@sdu.edu.cn (Z. Li).

0014-2999/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2012.01.011

Andrade et al., 2005; Lu et al., 2005; Shi et al., 2006). Mechanisms responsible for diabetic neuropathic pain are still unclear (Orstavik and Jorum, 2010; Yuan et al., 2009). A proper understanding of the mechanisms underlying diabetic neuropathic pain is highly important for treatment of painful diabetic neuropathy through an expanding repertoire of effective pharmacologic agents. The expression of galanin in DRG and SDH and the role of galanin in the regulation of pain transmission in diabetic neuropathy remain unknown. In the present study, the expression of galanin and its receptors 1 and 2 in DRG and SDH in diabetic rats was detected, and the effects of exogenous galanin, galanin receptor antagonist, selective galanin receptor 1 agonist, and selective galanin receptor 2 agonist on neuropathic pain in diabetic rats were evaluated.

2. Materials and methods

2.1. Diabetic neuropathic pain animal model

All preparations utilized male rats (200 g–250 g) taken from the breeding colony of Wistar rats maintained in the Experimental Animal Center at Shandong University of China. The present study was conducted following a protocol approved by the ethical committee of Shandong University and in agreement with the guidelines recommended by the International Association for the Study of Pain for experimental pain in conscious animals (Zimmermann, 1983). The rats were housed in plastic cages with a normal light–dark cycle and allowed free access to rat chow and water. Diabetes was induced in Wistar rats (after an overnight fast) by a single intraperitoneal injection (i.p.) of 55 mg/kg streptozotocin freshly dissolved in 0.1 mol/l citric acid buffer (pH 4.5). Age-matched control rats received an injection of equivalent volume citrate buffer alone. The serum glucose level was assayed by blood-glucose meter at 3 days after streptozotocin injection and at the time prior to experiment completion. Diabetes was confirmed in streptozotocin injected rats with blood glucose concentrations greater than 20 mmol/l. The body weight of the rats was monitored weekly. After 4 weeks of diabetes, only the rats with evident hyperalgesia in test of von Frey filaments (50% withdrawal threshold <5 g, see Section 2.3.) were included in the study.

2.2. Catheter implantation and intrathecal drug administration

For intrathecal injection (i.t.), the rat was anesthetized with of 10% chloral hydrate (300 mg/kg, i.p.). A sterile polyethylene catheter (PE-10, 8.0 cm in length) (Instech Laboratories Incorporation, Plymouth Meeting, Plymouth Meeting, PA, USA) was inserted through an incision in the gap between the L3/L4 vertebrae and stretched 1 cm to 1.5 cm cephalad so that the tip of catheter was positioned at the subarachnoid space of the lumbar enlargement in rat. The intrathecal catheter was externalized and fixed to the back of the neck. After a 3-day recovery period and at the end of the experiment, 2% lidocaine (10 μl, i.t.) was given to make sure the catheter was in appropriate position. Only the rat showing reversible hind limb motor deficits immediately following the lidocaine injection was considered to be catheterized successfully. All the drugs were dissolved in saline and given in the volume of 10 μl, followed 10 μl saline flushing the catheter.

2.3. Evaluation of mechanical hyperalgesia

Rats were placed in a testing cage with a wire mesh bottom and allowed to acclimate for 30 min before beginning the tests. The test of von Frey filaments was used to determine the 50% threshold for foot withdrawal. A series of filaments (0.1, 0.5, 1.0, 3.0, 3.8, 5.0, 8.2, and 14.6 g), starting with 3.0 g, were applied to the midplantar surface of each hind paw for 5 s with a pressure that was just sufficient to bend the filament. A brisk withdrawal of the paw when applying of a von Frey filament was recorded as a positive response. A withdrawal response led to use of the next weaker filament, and lack of withdrawal led to presentation of the next stiffer filament. Stimuli were presented at intervals of at least 5 s. The score was cut off at a force of 14.6 g to prevent the hind paw from damage. Interpolation of the 50% threshold was carried out according to the sequence of positive and negative scores. Each hind paw score was recorded twice for average.

2.4. Western blot assay of galanin, galanin receptor 1, and galanin receptor 2

At the designed experimental time (6 weeks after streptozotocin administration), the L4-5 DRG and the corresponding SDH of diabetic rats were surgically harvested. The expression of galanin, galanin receptor 1, and galanin receptor 2 was detected by Western blot assay. The DRGs were homogenized in 10 mmol/l Tris homogenization buffer (pH 7.4) with protease inhibitors (Amersco). The samples were centrifuged at 10,000 g for 10 min and the supernatant collected for Western blot. After determining the protein concentrations of the supernatants (BCA method, standard: BSA), 50 μg protein of each sample was loaded onto the 10% SDS gel, separated by electrophoresis and transferred to nitrocellulose membrane. The membranes were blocked in blocking buffer (5% nonfat milk) for 2 h at room temperature, and then were incubated with goat anti-galanin polyclonal IgG (1:500, Santa Cruz Biotechnology, Galanin (N-20)), goat anti-galanin receptor 1 polyclonal IgG (1:500, Santa Cruz Biotechnology, Galanin receptor 1 (C-20)), goat anti-galanin receptor 2 polyclonal IgG (1:500, Santa Cruz Biotechnology, Galanin receptor 2 (L-20)) or mouse anti-β-actin monoclonal IgG (1:1000, Santa Cruz Biotechnology, β-actin(C4)) overnight at 4 °C. After being washed 3 times for 10 min with washing solution, the membranes were incubated with rabbit anti-goat IgG-HRP (1:4000, Santa Cruz Biotechnology) or goat anti-mouse IgG-HRP (1:4000, Santa Cruz Biotechnology). The immunoreactive bands were visualized by an ECL Western blotting detection kit (Billerica) on light sensitive film. The films were scanned and the images were analyzed quantitatively by using an ImagJ 1.39u image analysis software. The levels of galanin, galanin receptor 1, and galanin receptor 2 were expressed as the ratio of the protein to β-actin.

2.5. Evaluation of the effects of galanin, M35, M617, and AR-M1896

After 6 weeks of streptozotocin administration, both diabetic and non-diabetic rats received interventions. Six diabetic rats were administered galanin (Tocris Bioscience) at a dose of 30 μg (i.t.). Six diabetic rats were given M35 (Bachem), galanin(1-13)-bradykinin(2-9)amide, a galanin receptor antagonist, at a dose of 2 μg (i.t.) 15 min before galanin administration. To further confirm whether galanin receptor 1 was involved in this effect, selective galanin receptor 1 agonist M617 (Tocris Bioscience), galanin(1-13)-Gln14-bradykinin(2-9)amide, at a dose of 10 μg (i.t.) was administered to 6 diabetic rats. To further confirm whether galanin receptor 2 was involved in this effect, selective galanin receptor 2 agonist AR-M1896 (Tocris Bioscience), galanin(2-11)amide, at a dose of 20 μg (i.t.) was administered to 6 diabetic rats. Another 6 diabetic rats were administered the same volume of saline. Six normal control animals were administered the same volume of saline. After that, the paw withdrawal threshold to mechanical stimuli was measured for all the experimental animals.

2.6. Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was evaluated with SPSS software by one-way ANOVA followed by the Student-Newman–Keuls test for significance to compare the differences among various groups or two independent sample *t*-test for significance to compare the difference between two groups. Significance was accepted at $P<0.05$.