Neuroscience Research 106 (2016) 47–54

Contents lists available at ScienceDirect

Neuroscience Research

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

Impaired expression of neuronal nitric oxide synthase in the gracile nucleus is involved in neuropathic changes in Zucker Diabetic Fatty rats with and without 2,5-hexanedione intoxication

Sheng-Xing Ma[a,*], Richard G. Peterson[b], Edward M. Magee[a], Paul Lee[a], Wai-Nang Paul Lee[c], Xi-Yan Li[a]

[a] Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center and Department of Obstetrics and Gynecology, David Geffen School of Medicine at University of California Los Angeles and Harbor-UCLA Medical Center, 1124 W. Carson Street, CA 90502, United States
[b] Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN 46202-5120, United States
[c] Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center and Department of Pediatrics, David Geffen School of Medicine at University of California Los Angeles and Harbor-UCLA Medical Center, 1124 W. Carson Street, CA 90502, United States

ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 10 October 2015
Accepted 16 October 2015
Available online 28 October 2015

Keywords:
Nitric oxide synthase
Dorsal medulla
Diabetic neuropathy
Sensory functions
2,5-HD intoxication
Immunohistochemistry

ABSTRACT

These studies examined the influence of 2,5-hexanedione (2,5-HD) intoxication on expression of neuronal nitric oxide synthase (nNOS) in the brainstem nuclei in Zucker Diabetic Fatty (ZDF) vs. lean control (LC) rats. Functional neuropathic changes were also investigated following axonal damage and impaired axonal transport induced by the treatment. Animals were intoxicated by i.p. injection of 2,5-HD plus unilateral administration of 2,5-HD over the sciatic nerve. The mechanical thresholds and withdrawal latencies to heat and cold stimuli on the foot were measured at baseline and after intoxication. The medulla sections were examined by nNOS immunohistochemistry and NADPH-diaphorase histochemistry at the end of the treatments. The mechanical thresholds and withdrawal latencies were significantly decreased while nNOS immunostained neurons and NADPH-diaphorase positive cells were selectively reduced in the gracile nucleus at baseline in ZDF vs. LC rats. NADPH-diaphorase reactivity and nNOS positive neurons were increased in the ipsilateral gracile nucleus in LC rats following 2,5-HD intoxication, but its up-regulation was attenuated in ZDF rats. These results suggest that diabetic and chemical intoxication-induced nNOS expression is selectively reduced in the gracile nucleus in ZDF rats. Impaired axonal damage-induced nNOS expression in the gracile nucleus is involved in neuropathic pathophysiology in type II diabetic rats.

1. Introduction

Unremitting pain and reduced temperature and vibration thresholds are observed in patients with diabetic neuropathy (Dyck et al., 1993), and in rats with streptozotocin-induced type I diabetes (Wuarin-Bierman et al., 1987). Recent studies showed that pain and pressure thresholds are decreased in Zucker Diabetic Fatty (ZDF) rats, a type II diabetic model (Rong and Ma, 2011; Russell et al., 2008; Zhuang et al., 1997). Several studies demonstrated that systemic administration of 2,5-hexanedione (2,5-HD) causes neurofilamentous axonal swellings and damages, which prevent proximal-to-distal transport of neurofilaments and other substances, resulting in profound neuropathic changes including hyperalgesia and limb paralysis (Anthony et al., 1983; LoPachin et al., 1994). However, the neural pathways and neurotransmissions responsible for the increased susceptibility of the sensory neurons to non-noxious and noxious stimuli in diabetic and chemical neuropathies are poorly understood.

Nitric oxide (NO) is one of the most important messenger molecules produced in many cell types, including neurons in the brain (Bredt and Snyder, 1992; Moncada and Higgs, 1991). Several

Abbreviations: 2,5-HD, 2,5-hexanedione; LC, lean control; NO, nitric oxide; NADPHd, NADPH diaphorase; nNOS, neuronal nitric oxide synthase; ZDF, Zucker Diabetic Fatty.

* Corresponding author at: LA BioMed at Harbor-UCLA Medical Center and Department of Ob/Gyn, David Geffen School of Medicine at UCLA, Harbor-UCLA Medical Center, 1124 W. Carson Street, RB-1, Torrance, CA 90502, United States. Tel.: +1 310 222 1964; fax: +1 310 222 4131.
E-mail address: S.ma@ucla.edu (S.-X. Ma).

http://dx.doi.org/10.1016/j.neures.2015.10.007
0168-0102/

studies have shown that the nitric oxide (NO)–cGMP pathway plays an inhibitory role in nociceptive modulation, which contributes to analgesic mechanisms (Duarte et al., 1990; Kumar et al., 1993). Recent studies show that impaired NO production is involved in human diabetic neuropathy (Kilo et al., 2000) and is associated with hyperalgesia in diabetic rats (Sessa et al., 1993). nNOS catalyses the transformation of arginine to NO in neurons, and nNOS is also a highly regulated enzyme (Dinerman et al., 1990; Sessa et al., 1993). Recent studies have demonstrated that nNOS is an inducible enzyme, which is up-regulated by lesion of nerves, abnormal mechanical forces, and other various factors (Amin et al., 1995; Dinerman et al., 1990; Ma et al., 2000; Sessa et al., 1993). nNOS expression in the gracile nucleus is markedly increased in rats with sciatic axotomy and accompanied by an increased number of cells showing expression of NADPH diaphorase (NADPHd) reactivity, a marker of nNOS (Ma et al., 2000).

The gracile nucleus receives ascending input from the sciatic nerve, which has the longest axons in the body (Leem et al., 1994; Ueyama et al., 1994). Recent experiments have suggested that the gracile nucleus is an integration center for visceral and somatic information flowing into the thalamus, which possesses functions for sensory and pain processing in the dorsal column pathway (Al-Chaer et al., 1996, 1997). The afferent sensory fibers in the sciatic nerve originate from the skin or muscle, and synapse directly on dorsal horn neurons, or on dorsal horn interneurons in the spinal cord, which ascends to the gracile nucleus (Leem et al., 1994; Ueyama et al., 1994). Previous studies have demonstrated that neuropeptide Y and substance P immunoreactivities increase in the gracile nucleus after sciatic nerve damage or transection (Noguchi et al., 1995; Ohara et al., 1994; Zhang et al., 1993).

The purpose of the present study was to determine the influence of 2,5-HD intoxication, which induces axonal damage and impaired axonal transport, on nNOS expression in the brainstem and on functional neuropathic changes in ZDF rats compared to normal lean control (LC) rats. Baseline and 2,5-HD intoxication-induced nNOS expressions in the brainstem nuclei were examined by using nNOS immunohistochemistry and NADPHd histochemistry, a marker of nNOS activity. Functional neuropathic changes were examined by measuring the mechanical tolerance threshold of the foot using Von Frey Filaments and by testing the withdrawal latencies of the foot in response to heat and cold stimuli.

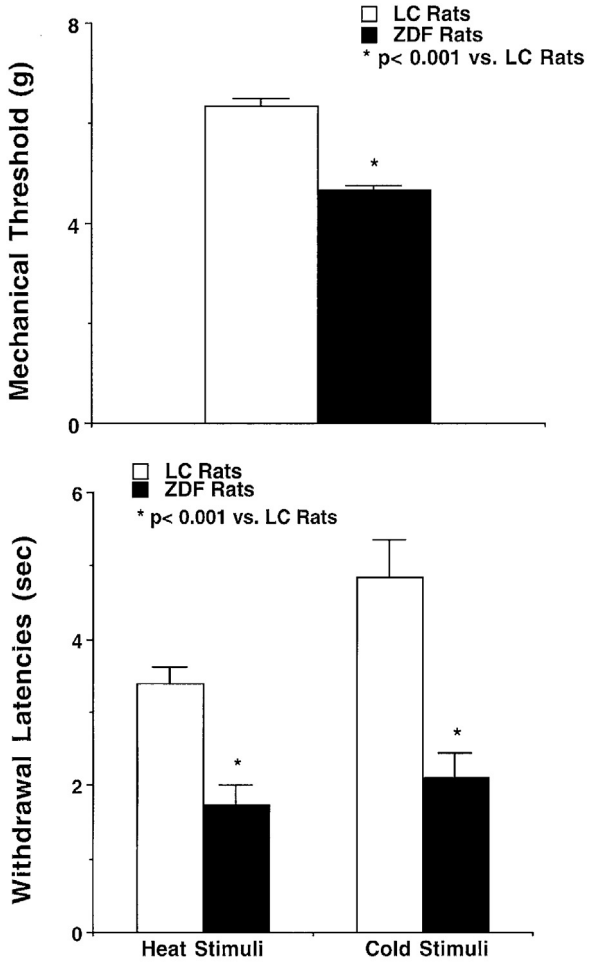

Fig. 1. Foot-mechanical thresholds and withdrawal latencies to heat and cold stimuli in ZDF rats vs. lean control (LC) rats. Mechanical thresholds in the ZDF rat are significantly lower than that in the LC rat (top panels). Bottom panels show that ZDF rats have a markedly faster foot withdrawal to heat (left) and cold (right) stimuli compared to LC rats. Values are mean ± SEM (n = 7/group). *P < 0.001, compared with LC rats.

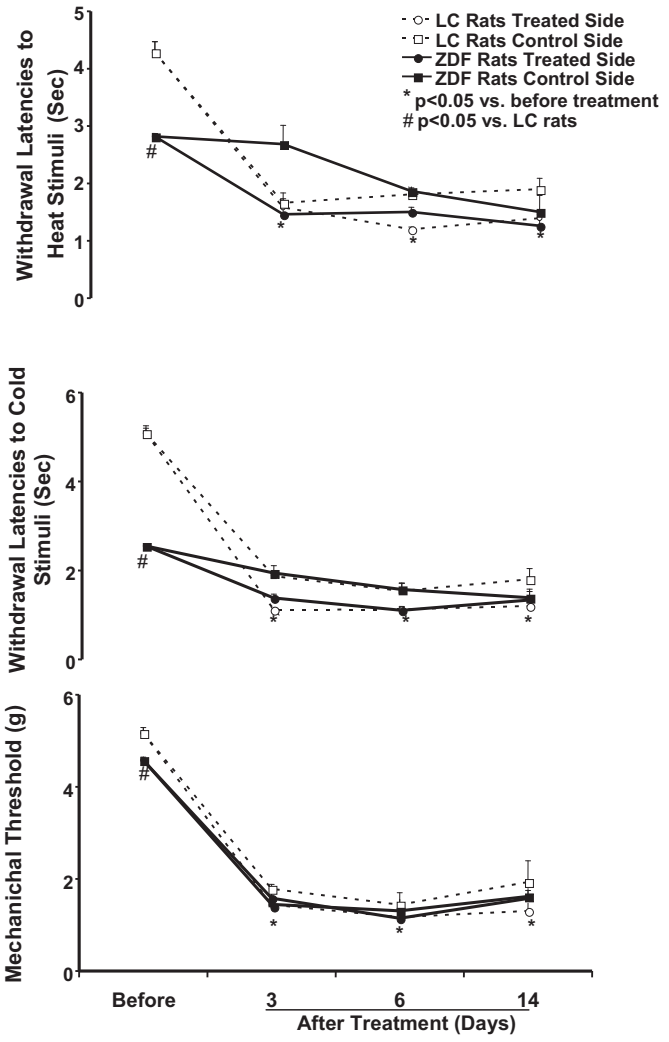

Fig. 2. The time intervals of changes in withdrawal latency responses to heat and cold stimuli (top and middle panels) and mechanical threshold (bottom panels) on the control and treated sides of foot induced by 2,5-hexanedione (2,5-HD) intoxication in ZDF rats compared to lean control (LC) rats. Withdrawal latency responses to heat and cold stimuli and mechanical threshold showed significant decreases in the control value of ZDF rats compared to LC rats. Mechanical tolerance thresholds and withdrawal latencies to heat and cold stimuli were significantly decreased on both sides of the foot at 3, 6, and 14 days after 2,5-HD treatments in ZDF and LC rats. Values are mean ± SEM (n = 5–6/group). *P < 0.05, compared with before treatment; #P < 0.05, compared with LC rats.