Life Sciences 90 (2012) 278–288

Contents lists available at SciVerse ScienceDirect

Life Sciences

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

Decreases of glycine receptor expression induced by median nerve injury in the rat cuneate nucleus contribute to NPY release and c-Fos expression

Seu-Hwa Chen [a,b], Yi-Ju Tsai [c], Hsin-Ying Wang [a], Chi-Te Lin [a], Shin-Fang Li [a], June-Horng Lue [a,*]

[a] Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei, Taiwan
[b] Department of Anatomy, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[c] School of Medicine, College of Medicine, Fu Jen Catholic University, New Taipei, Taiwan

ARTICLE INFO

Article history:
Received 13 June 2011
Accepted 23 November 2011

Keywords:
Transection
Fluorogold
Cuneothalamic projection neuron
Glycine
Electrical stimulation
CatWalk

ABSTRACT

Aims: This study aimed to investigate temporal changes in glycine and its receptor expressions in cuneate neurons after median nerve transection (MNT), and the effects of glycine on neuropeptide Y (NPY) release and c-Fos expression in the cuneate nucleus (CN).
Main methods: Immunohistochemistry methods were used to appraise changes of glycine- and GlyR-like immunoreactive (LI) neurons in the CN after MNT. The alterations in NPY and c-Fos expressions were used to assess the effects of saline, glycine or strychnine treatment. The CatWalk method was used to assess the efficiency of glycine treatment on the neuropathic signs of rats with MNT.
Key findings: Approximately half of GlyR-LI neurons were fluorogold-labeled cuneothalamic projection neurons in the CN. Following MNT, the number of GlyR-LI neurons significantly decreased in the injured side of CN at 2 and 4 weeks, but the number of glycine-LI neurons remained unchanged. Four weeks after MNT given with electrical stimulation, strychnine significantly decreased the NPY reduction level in the stimulated side CN compared to that of the saline group. However, numbers of c-Fos-LI neurons in the glycine and strychnine groups were both significantly less than that in the saline group. But the paw print width and area in CatWalk analysis showed only a moderate recovery.
Significance: We conjecture that glycine increases glycine-mediated postsynaptic inhibition of cuneate neurons, and also blocks GABAergic neurons containing GlyRs which mediate presynaptic inhibition causing temperate NPY release. Consequently, the compromise results showed a weak reduction in c-Fos expression and a slight amelioration of neuropathic behaviors.

Introduction

In the cuneate nucleus (CN), glycine is well known to be involved in postsynaptic inhibition of cuneothalamic projection neurons (CTNs) (Davidson and Southwick, 1971; Kelly and Renaud, 1973). An ultrastructural study further provided direct morphological evidence that glycine-like immunoreactive (glycine-LI) terminals make axosomatic and axodendritic synapses with CTNs (Lue et al., 2000). However, the topographic distribution of the glycine receptor (GlyR) in the CN and its characterization need to be explored. According to previous studies, the putative neuroelements for glycine receptor-like immunoreactive (GlyR-LI) might be derived from CTN, γ-aminobutyric acid (GABA)-LI, and glycine-LI neurons (Lue et al., 2000, 2001). Under normal conditions, the CN modulates and transmits innocuous perceptions mainly from the forelimb via dorsal root ganglion neurons and their primary afferent fibers and then transfers the message to the contralateral ventrobasal nucleus of the thalamus. On the other hand, following median nerve injury, neuropathic pain develops on the injured forepaw, and neuropeptide Y (NPY) and c-Fos are both de novo-expressed in the ipsilateral CN (Day et al., 2001; Saadé and Jabbur, 2008; Tsai et al., 2009). The expression of c-Fos, a protein product of the immediate-early proto-oncogene, *c-fos*, is universally used to identify populations of neurons that are activated by noxious stimuli, and is regarded as a neural marker of pain (Hunt et al., 1987; Harris, 1998). With injury to the median nerve, the decline in inhibitory modulation might be one of the factors causing hyperexcitability and Fos expression in cuneate neurons for putative neuropathic pain transmission. Sciatic nerve injury was subsequently associated with dramatic decreases in the number of GABA neurons and $GABA_B$ receptors binding in the spinal dorsal horn, and thermal hyperalgesia and allodynia coincidentally developed (Castro-Lopes et al., 1993, 1995; Moore et al., 2002; Scholz et al., 2005). With chronic constriction injury to the sciatic nerve, there was no apparent change in the number of glycine neurons in the dorsal horn (Polgar et al., 2003). However, there is a lack of evidence related to the effect of median nerve transection (MNT) on

* Corresponding author at: Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, 1-1 Jen Ai Rd. Taipei 10018, Taiwan Tel.: +886 2 23123456 ext. 88178; fax: +886 2 23955804.
E-mail address: thomas@ntu.edu.tw (J.-H. Lue).

0024-3205/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2011.11.014

changes in numbers of glycine-LI and GlyR-LI neurons in the CN, which may further contribute to corresponding neuropathic signs.

Using GlyR immunohistochemistry, this study first investigated the topographic distribution of GlyR-LI neurons and their ultrastructures in the CN. Combining retrograde fluorogold (FG) labeling and immunofluorescence method, we sought to identify whether GlyR-LI neurons are CTNs, GABA-LI neurons, or glycine-LI neurons. Since GlyR-LI neurons are predominantly localized in the middle CN and are mainly derived from CTNs, we examined whether numbers of GlyR-LI, double-labeled (GlyR + CTN), and glycine-LI neurons in the CN were modified at 2 and 4 weeks after MNT. Furthermore, we evaluated whether intraperitoneal application of glycine receptor agonist (glycine) and antagonist (strychnine) before electrical stimulation of the injured median nerve regulated NPY release and c-Fos expression in the CN. If c-Fos expression in the CN was reduced by glycine, we attempted to examine whether glycine treatment could ameliorate neuropathic pain behaviors by a CatWalk method analysis.

Materials and methods

Animal preparations

These experiments were approved by the institutional review board, and were conducted in accordance with the guidelines for the care and use of laboratory animals of the Animal Care and Use Committee (IACUC), College of Medicine and College of Public Health, National Taiwan University Taiwan (IACUC approval no. 20080267). The guidelines on ethical standards for investigations of experimental pain in animals were followed (Zimmermann, 1983). Animals were divided into five groups (groups 1–5; Fig. 1) as described here. They were housed under approved conditions with a 12/12-h light/dark cycle, and food and water were provided ad libitum.

Nerve injury surgery

For the experimental design, animals in groups 2–5 were individually given sham surgery, unilateral or bilateral MNT (Fig. 1). Animals were anesthetized with 7% chloral hydrate intraperitoneally (0.45 ml/100 g body weight), and their median nerves were carefully isolated from the surrounding tissue at the level of the elbow immediately proximal to where the nerve entered between the two heads of the pronator teres muscle. A tight ligature (5.0 silk) was made around the nerve, and about a 2-mm segment of the distal end was cut and removed (Lue et al., 2002; Lin et al., 2009; Tsai et al., 2009). Following surgery, the wound was sutured. In the naïve group (group 1), the forelimb was left intact. For the sham surgeries, the median nerve was exposed at the same level of the forelimb but was not transected. Animals that were operated on were allowed to recover for 2 or 4 weeks after MNT. The weights and forelimb externals of these animals did not differ among groups before perfusion.

Combined fluorogold (FG) tracing and GlyR immunohistochemistry

Three days prior to sacrifice, rats in groups 1–3 that had or had not undergone unilateral MNT were used for retrograde labeling of CTNs by 1 μl of 2% FG (Fluorochrome, Denver, CO, USA) injected stereotactically into the contralateral ventrobasal nucleus of the thalamus (Pellegrino et al., 1979; Day et al., 2001; Lue et al., 2002; Tsai et al., 2009) (coordinates AP, ML, H: 4, 3, −0.5; 4, 3, 0; 4, 2.5, −1; System A). Two or four weeks after MNT, these rats were re-anesthetized and perfused with a mixed solution consisting of 2.5% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M phosphate buffer (PB) (pH 7.4). Tissue blocks of the medulla containing the CN were removed and further fixed in the same fixative for 2 h after perfusion. Then, they were cut transversely with a vibratome (TPI, Portland, OR, Series 1000, St. Louis, MO, USA) at 30 μm thick. Serial sections were collected from the entire rostrocaudal extent of the CN and grouped into four sets. Sections of the medulla containing the CN were observed with an epifluorescence microscope to visualize FG and were photographed. Subsequently, these sections were subjected to GlyR immunolabeling. Two sets were treated with 3% H_2O_2 and blocked with 10% normal goat serum in phosphate-buffered saline for 1 h. Sections were incubated in 1:1000 rabbit anti-GlyR antiserum (AB5052, Chemicon, Millipore, Billerica, MA, USA) at 4 °C for 36 h, followed by

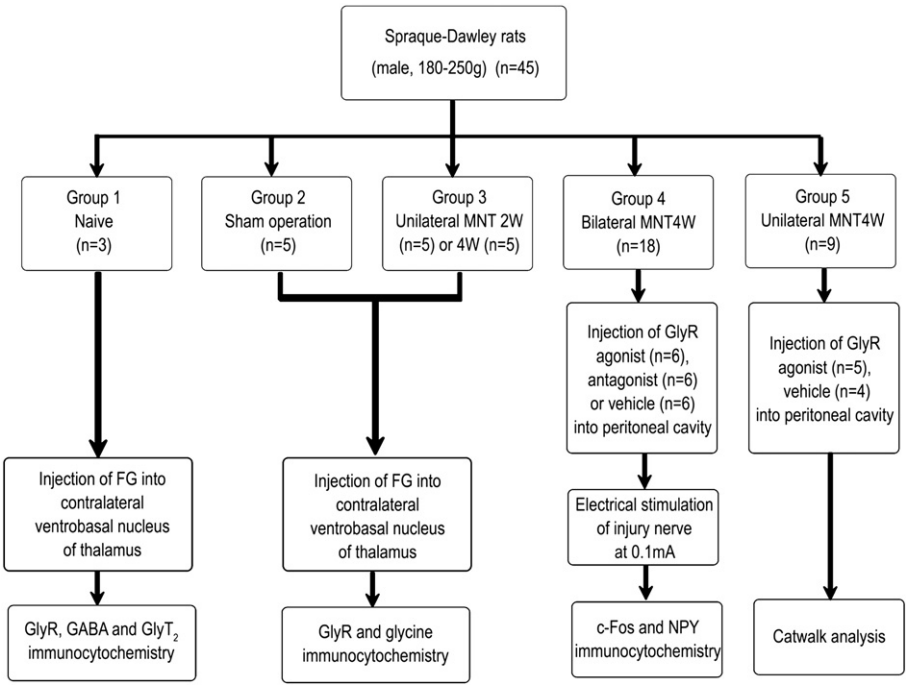

Fig. 1. Flow diagram of the MNT groups. MNT, median nerve transection; NPY, neuropeptide Y; FG, Fluorogold.