Experimental Neurology 276 (2016) 31–40

Contents lists available at ScienceDirect

Experimental Neurology

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

TrkB gene therapy by adeno-associated virus enhances recovery after cervical spinal cord injury

Gabriel Martínez-Gálvez [a,b,1], Juan M. Zambrano [a,b,1], Juan C. Diaz Soto [c], Wen-Zhi Zhan [a], Heather M. Gransee [a], Gary C. Sieck [a,c], Carlos B. Mantilla [a,c,*]

[a] Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN 55905, United States
[b] Department of Biomedical Engineering, Universidad de los Andes, Bogotá, Colombia
[c] Department of Anesthesiology, Mayo Clinic, Rochester, MN 55905, United States

ARTICLE INFO

Article history:
Received 6 August 2015
Received in revised form 12 November 2015
Accepted 18 November 2015
Available online 1 December 2015

Keywords:
Neurotrophin
Neuroplasticity
Spinal hemisection
Diaphragm muscle
Phrenic motoneuron
Respiratory
Glutamatergic
Serotonergic
Neurotransmitter

ABSTRACT

Unilateral cervical spinal cord hemisection at C2 (C2SH) interrupts descending bulbospinal inputs to phrenic motoneurons, paralyzing the diaphragm muscle. Recovery after C2SH is enhanced by brain derived neurotrophic factor (BDNF) signaling via the tropomyosin-related kinase subtype B (TrkB) receptor in phrenic motoneurons. The role for gene therapy using adeno-associated virus (AAV)-mediated delivery of TrkB to phrenic motoneurons is not known. The present study determined the therapeutic efficacy of intrapleural delivery of AAV7 encoding for full-length TrkB (AAV-TrkB) to phrenic motoneurons 3 days post-C2SH. Diaphragm EMG was recorded chronically in male rats ($n = 26$) up to 21 days post-C2SH. Absent ipsilateral diaphragm EMG activity was verified 3 days post-C2SH. A greater proportion of animals displayed recovery of ipsilateral diaphragm EMG activity during eupnea by 14 and 21 days post-SH after AAV-TrkB (10/15) compared to AAV-GFP treatment (2/11; $p = 0.031$). Diaphragm EMG amplitude increased over time post-C2SH ($p < 0.001$), and by 14 days post-C2SH, AAV-TrkB treated animals displaying recovery achieved 48% of the pre-injury values compared to 27% in AAV-GFP treated animals. Phrenic motoneuron mRNA expression of glutamatergic AMPA and NMDA receptors revealed a significant, positive correlation ($r^2 = 0.82$), with increased motoneuron NMDA expression evident in animals treated with AAV-TrkB and that displayed recovery after C2SH. Overall, gene therapy using intrapleural delivery of AAV-TrkB to phrenic motoneurons is sufficient to promote recovery of diaphragm activity, adding a novel potential intervention that can be administered after upper cervical spinal cord injury to improve impaired respiratory function.

1. Introduction

Unilateral spinal cord hemisection at C2 (C2SH) interrupts descending premotor bulbospinal drive to phrenic motoneurons located between C3–C6 segments of the spinal cord in rats, resulting in transient paralysis of the ipsilateral diaphragm muscle (DIAm) (Goshgarian, et al., 1991, Mantilla, et al., 2013a,b, Porter, 1895). Over time, spontaneous recovery of ipsilateral rhythmic DIAm activity ensues, reflecting neuroplasticity and strengthening of spared synaptic input to phrenic motoneurons (Fuller, et al., 2006, Golder, et al., 2003, Mantilla, et al., 2012, Mantilla, et al., 2013a,b, Nantwi, et al., 1999, O'Hara and Goshgarian, 1991). This model has been widely used and provides a well validated and useful tool for investigating the mechanisms underlying recovery after incomplete upper cervical spinal cord injury (Alilain and Goshgarian, 2008, Alilain, et al., 2011, Golder and Mitchell, 2005, Mantilla, et al., 2013a,b, Mantilla and Sieck, 2009, Rowley, et al., 2005, Sieck and Mantilla, 2009, Zhan, et al., 1997).

The importance of brain-derived neurotrophic factor (BDNF) in synaptic plasticity is well documented (Bregman, et al., 2002, Coumans, et al., 2001, Friedman, et al., 1995, Kang and Schuman, 1995, Poo, 2001, Thoenen, 1995). Recent studies highlight the role of BDNF acting through its high-affinity tropomyosin-related kinase type B (TrkB) receptor in promoting recovery of DIAm EMG activity following C2SH (Gransee, et al., 2013, Mantilla, et al., 2013a, Mantilla, et al., 2014). Intrathecal administration of BDNF (Mantilla, et al., 2013a), as well as increased TrkB receptor expression induced by adeno-associated virus (AAV)-mediated targeted delivery of full-length TrkB to phrenic motoneurons prior to the injury (Gransee, et al., 2013) indicate that enhancing BDNF/TrkB signaling in phrenic motoneurons promotes recovery of ipsilateral DIAm activity following C2SH. Conversely, blocking BDNF/TrkB signaling through TrkB siRNA-mediated knockdown or chemical-genetic inhibition of TrkB kinase activity abrogates spontaneous recovery after C2SH (Mantilla, et al., 2013a, Mantilla, et al., 2014). At present,

* Corresponding author: 200 First Street SW, 4-184 W. Joseph SMH, Rochester, MN 55905, United States.
E-mail address: mantilla.carlos@mayo.edu (C.B. Mantilla).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.expneurol.2015.11.007
0014-4886/

the therapeutic role of increasing TrkB receptor expression in phrenic motoneurons following injury has not been assessed. In particular, it is important to disambiguate the possible contribution of plasticity resulting from pre-injury AAV treatment targeting phrenic motoneurons (e.g., a conditioning effect) and/or changes in synaptic inputs to motoneurons that increase resiliency to subsequent injury. We hypothesized that targeted, viral-mediated delivery of TrkB to phrenic motoneurons is sufficient to enhance recovery of ipsilateral DIAm activity following upper cervical spinal cord injury.

Several studies indicate that expression of glutamatergic (GluR) and serotonergic (5-HTR) receptors (specifically NMDA and 5-HTR2a, respectively) increases over time after C2SH and the timing of changes in expression generally corresponds with the onset of spontaneous recovery of ipsilateral phrenic activity (Alilain and Goshgarian, 2008, Fuller, et al., 2005, Mantilla, et al., 2012). Neurotrophins such as BDNF influence post-synaptic expression of excitatory neurotransmitter receptors (Gottschalk, et al., 1999, Kang and Schuman, 1995) and increase motoneuron excitability (Gonzalez and Collins, 1997), at least in part via altered expression of GluR (Lessmann, et al., 1994) and 5-HTR receptors (Baker-Herman, et al., 2004). Accordingly, a secondary purpose of this study was to determine phrenic motoneuron expression of GluR and 5-HTR following targeted intrapleural delivery of AAV-TrkB or AAV-GFP. We hypothesized that phrenic motoneuron expression of GluR and 5-HTR increases following targeted delivery of AAV-TrkB to phrenic motoneurons, particularly in animals displaying recovery after C2SH.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (280-300 g; n = 26) were purchased from Harlan (Indianapolis, IN). Food and water were provided ad libitum for the duration of the study. Anesthesia for surgical procedures was conducted with intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). All of the experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee at Mayo Clinic, in compliance with the American Physiological Society guidelines.

Animals were randomly assigned to two groups: intrapleural injection of AAV-GFP (n = 11) or AAV-TrkB (n = 15) prior to placement of electrodes for chronic EMG recordings. DIAm EMG activity was used to verify the completeness of C2SH prior to AAV injection and to monitor recovery of rhythmic ipsilateral activity over time (7, 14 and 21 days post-C2SH). At the terminal experiment rats were euthanized by exsanguination and the spinal cord was dissected under RNase free conditions and frozen for further analysis.

2.2. Electrode implantation

Implantation of chronic DIAm electrodes was performed in all rats at ~3 days prior to C2SH, as previously described (Dow, et al., 2006, Dow, et al., 2009, Gransee, et al., 2013, Gransee, et al., 2015, Mantilla, et al., 2013a,b, Mantilla, et al., 2011, Trelease, et al., 1982). Briefly, a 2 mm segment of insulated stainless steel wire (AS631, Cooner Wire Inc., Chatsworth, CA) was stripped of insulation and inserted into the midcostal region of the DIAm. Two electrodes were inserted in each side and externalized for chronic EMG recordings.

2.3. Spinal hemisection (C2SH)

Spinal hemisection was conducted as previously detailed and validated (Gransee, et al., 2013, Gransee, et al., 2015, Mantilla, et al., 2012, Mantilla, et al., 2014, Mantilla, et al., 2013a,b, Mantilla, et al., 2007, Miyata, et al., 1995, Prakash, et al., 1999). Using a dissecting microscope under sterile conditions a dorsal C2 laminectomy was performed and only the right anterolateral spinal cord was transected at C2 with a microknife. Animals received analgesics for the first three days postoperatively, including oral acetaminophen (100–300 mg/kg) and intramuscular buprenorphine (0.1 mg/kg) as needed. Complete hemisection was verified by absence of ipsilateral DIAm EMG activity immediately following the procedure and 3 days following C2SH.

2.4. Chronic EMG recordings and analysis

In anesthetized animals, DIAm EMG recordings were conducted during eupnea at various time points: prior to C2SH on day 0 and at 3, 7, 14 and 21 days post-C2SH, in accordance with previous studies (Gransee, et al., 2013, Gransee, et al., 2015, Mantilla, et al., 2013a,b, Mantilla, et al., 2013a,b, Mantilla, et al., 2011). Eupneic recordings were conducted for at least 2 min in lightly anesthetized animals (using ~one-third of the surgical anesthesia dose) while the animals were supine resting on a heating pad. At day 21, EMG recordings were additionally conducted during exposure to hypoxic (10% O_2)-hypercapnic (5% CO_2) conditions for 5 min and during sustained airway occlusion for 45 s. Data acquisition was conducted using LabView software (National Instruments, Austin, TX) at a sampling frequency of 2 kHz, following filtering (20–1000 Hz bandpass) and amplification (×2000). Root mean square (RMS) DIAm EMG activity was measured using a 50 ms window as in previous studies (Mantilla, et al., 2011, Mantilla, et al., 2010). Digitized EMG signals were analyzed using MatLab 8.2 (MathWorks, Natick, MA). The mean peak RMS EMG activity was measured for eupnea (2 min), hypoxia–hypercapnia (last 30 s) and airway occlusion (last 5 s). In addition, spontaneous deep breaths ("sighs"), defined as inspiratory events with amplitude at least twice eupneic amplitude, were identified during periods of eupnea and hypoxia–hypercapnia pre-C2SH.

Recovery of ipsilateral DIAm EMG activity during eupnea was defined by the following criteria: 1) rhythmic DIAm EMG signal reflecting inspiratory activity in phase with contralateral DIAm activity across most inspiratory bursts (>90%), 2) DIAm EMG signal comprising more than one motor unit (multiple units with different waveform profiles), and 3) DIAm EMG amplitude at least 10% of pre-C2SH, consistent with previous studies (Dow, et al., 2009, Gransee, et al., 2013, Gransee, et al., 2015, Mantilla, et al., 2013a,b). The proportion of animals meeting criteria for recovery was determined at various time points following C2SH. In addition, peak RMS EMG amplitude was normalized to the mean peak RMS EMG pre-C2SH during eupnea for the same animal (normalized RMS EMG) in order to calculate the extent of recovery of ipsilateral DIAm EMG activity post-C2SH. Respiratory rate and duty cycle were calculated from contralateral DIAm EMG recordings at days 0 and 21 post-C2SH.

2.5. AAV administration

AAV serotype 7 vectors encoding human GFP (AAV.CMV.PI.EGFP.WPRE.bGH) or TrkB.FL (AAV7.CMV.Flag-TrkB.WPRE.bGH) under a CMV promoter were obtained from the Vector Core at University of Pennsylvania, as previously reported (Gransee, et al., 2013). At day 3 post-C2SH, 1×10^{11} genome copies of the AAV7 vector (GFP or TrkB.FL) were administered into the right (ipsilateral) pleural space between the 7th and 8th ribs via 2 injections (50 μl total) using a Hamilton syringe, as previously reported (Gransee, et al., 2013, Mantilla, et al., 2009). Intrapleural AAV injection results in greater than 15% efficiency in transduction of ipsilateral phrenic motoneurons with no other neurons labeled in the cervical spinal cord (Gransee, et al., 2013). Specific transduction of phrenic motoneurons following intrapleural AAV injection was previously verified by GFP immunoreactivity, FLAG protein expression in cervical homogenate and TrkB mRNA expression in laser capture microdissected phrenic motoneurons (Gransee, et al., 2013).