Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 378 (2005) 18–21
Neuroscience Letters
www.elsevier.com/locate/neulet

Direct in vivo protein transduction into a specific restricted brain area in rats

Kyoko Owada-Makabe[a], Yuji Tsubota[a], Kazunori Yukawa[a], Nobuyuki Kakimoto[a], Xiang-Miang Liang[b], Masakazu Ichinose[b], Masanobu Maeda[a,*]

[a] *Department of Physiology, Wakayama Medical University School of Medicine, Wakayama, Japan*
[b] *Department of Internal Medicine, Wakayama Medical University School of Medicine, 811-1 Kimiidera, Wakayama-city, Wakayama 641-8509, Japan*

Received 20 September 2004; received in revised form 26 November 2004; accepted 1 December 2004

Abstract

Attempts at protein transduction into specific restricted brain areas have remained unsuccessful. We attempted targeted, direct in vivo protein transduction by microinjecting β-galactosidase (β-gal) with hemagglutinating virus of Japan envelope (HVJ-E) vector into the rat nucleus tractus solitarius (NTS). The medulla oblongata including the NTS was removed 6 h post-injection and cryostat sections were histochemically stained to detect β-gal enzymatic activity. β-gal-positive cells were present in these sections as was β-gal activity determined by colorimetric analysis. β-gal-positive cells were not present in the rats microinjected only β-gal protein without HVJ-E vector. Our findings suggest that direct in vivo protein transduction into specific restricted brain areas is possible. The type of targeted delivery system we present may have wide applications in the administration of therapeutic proteins to the central nervous system.

Keywords: Protein transduction; Protein therapy; Brain; Nucleus tractus solitarius; Microinjection; Non-viral vector

For the efficient in vivo delivery of proteins into cells, the delivered molecules must be very small. Schwarze et al. [9], who injected mice intraperitoneally with β-galactosidase (β-gal) protein fused to the protein transduction domain from the human immunodeficiency virus TAT protein, reported that biologically active fusion protein was delivered to all tissues, including brain tissue. Other proteins that possess the ability to transduce are Drosophila Antennapedia homeotic transcription factor and herpes-simplex-virus-1 DNA-binding protein VP22 [4,5,11]. The in vivo delivery of biologically active proteins into cells is a powerful therapeutic tool and a precise understanding of protein transduction will not only facilitate rational drug design but also improve the efficacy of in vivo experiments [10]. While there are some reports regarding protein transduction into the brain [1,3], attempts at protein transduction into specific restricted brain areas have been unsuccessful to date. In a rat model, we investigated the possibility of direct in vivo β-gal protein transduction into the specific restricted autonomic nucleus in the brainstem, the nucleus tractus solitarius (NTS). We used a microinjection technique and hemagglutinating virus of Japan envelope (HVJ-E) vector.

All animal experiments complied with the guidelines of the Physiological Society of Japan [12] and the guidelines for animal experiments of Wakayama Medical University.

Male Wistar rats (Kiwa Experimental Animals, Japan), weighing 290–330 g, were anesthetized with pentobarbital sodium (45 mg/kg, i.p.; supplemental doses (5 mg/kg, i.p.) were given when necessary). Their rectal temperature was maintained at 37 °C with a thermostatically controlled heating pad (ATB-100, Nihon Kohden, Japan). Each rat was placed in a stereotaxic instrument, and the skull was exposed. A glass micropipette (GC200F-10, CLARK Electromedical Instruments, UK) with an outside diameter of 20–50 μm was used for the microinjection of β-gal protein with HVJ-E vector and only β-gal protein without HVJ-E vector. Controls were microinjected with only HVJ-E vector. A micropipette

* Corresponding author. Tel.: +81 73 441 0623; fax: +81 73 441 0623.
E-mail address: masanobu@wakayama-med.ac.jp (M. Maeda).

0304-3940/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.12.017

was inserted into the medulla oblongata to identify the NTS region. The coordinates for the NTS mediating cardiovascular responses were: 0.5 mm rostral to the calamus scriptorius, 0.5 mm lateral to the midline, at a depth of 0.5 mm from the calamus scriptorius. The microinjection (β-gal solution 16.7 ng; 100 nl volume) was delivered unilaterally during a 1–2-s period. After 5 min the micropipette was withdrawn, the dura mater covered, and the skin sutured. After

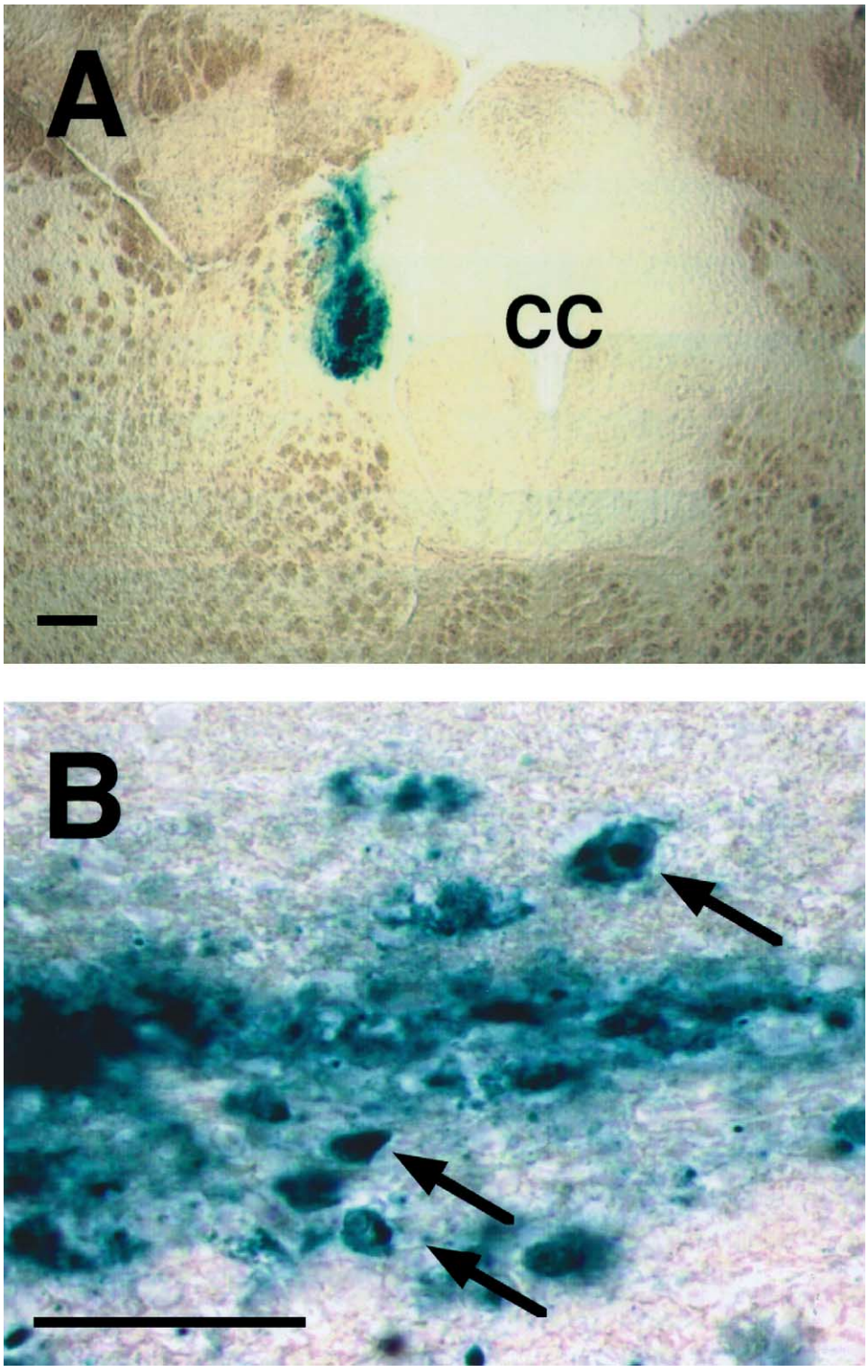

Fig. 1. Brain sections from rats microinjected with β-galactosidase (β-gal) protein using hemagglutinating virus of Japan envelope (HVJ-E) vector. Note the strong β-gal activity (A). X-Gal-stained neurons are seen at the site of microinjection (B). Arrows point to X-Gal-stained neuronal cells. CC, central canal. Scale bars: 100 μm.