Journal of Equine Veterinary Science 45 (2016) 28–31

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Short Communication

Cloning and Expression of Equine β-Nerve Growth Factor

Yosuke Amagai [a,1], Hiroaki Sato [a,1], Saori Ishizaka [a,b,1], Kenshiro Matsuda [a], Christine Aurich [b], Akane Tanaka [a,c], Hiroshi Matsuda [a,d,*]

[a] Cooperative Major in Advanced Health Science, Graduate School of Bio-Applications and System Engineering, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan
[b] Centre for Artificial Insemination and Embryo Transfer, University for Veterinary Sciences Vienna, Vienna, Austria
[c] Laboratory of Comparative Animal Medicine, Division of Animal Life Science, Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan
[d] Laboratory of Veterinary Molecular Pathology and Therapeutics, Division of Animal Life Science, Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

ARTICLE INFO

Article history:
Received 19 December 2015
Received in revised form 1 June 2016
Accepted 1 June 2016
Available online 9 June 2016

Keywords:
Nerve growth factor
Cloning
Horse

ABSTRACT

Nerve growth factor (NGF) is a neurotrophic factor that is essential for the maintenance of peripheral and central neurons. While importance of such physiological activities as well as the potential of it as a stress marker in *Equus caballus* has been implicated, the sequence of equine NGF remained unknown. In this study, we identified the sequence of equine NGF from the mRNA expressed in the peripheral blood in seven Thoroughbreds and three warmblood horses. There were no polymorphisms among samples analyzed, and the homology is more than 90% in comparison to human, mouse, rat, dog, and cow. When the sequence corresponding to the biologically active β-nerve growth factor was expressed in CHO-K1 cells, they were stained with an anti-NGF antibody that recognizes human NGF. Thus, it provides a rationale for using antibodies that react to other species' NGF to measure the equine NGF for further studies.

1. Introduction

Nerve growth factor (NGF) is a polypeptide necessary for the growth and support of neuron cells, which is produced from various kinds of cells such as hematopoietic cells, keratinocytes, and fibroblasts [1–4]. Besides nerve cell maintenance, it is involved in a wide variety of physiological activities such as pain sensation and wound healing [2–7]. In *Equus caballus*, NGF has been implicated as a novel stress marker because serum NGF levels elevated after running exercise or truck transportation [8,9]. In those studies, we estimated the elevation of NGF in an in vitro assay using rat PC12 pheochromocytoma cell line based on the premise that equine NGF sequence is highly conserved and crossreacts among species, leaving the sequence of equine NGF remained undetermined. Recently, Wade et al [10] provided a predicted sequence of equine NGF from a shotgun sequence analysis of a Thoroughbred genome. It indicates that equine NGF shares high homology with other species, though more data are required to conclude the equine NGF sequences taking polymorphisms into account.

In this study, we identified a sequence of equine NGF using blood samples from 10 horses and analyzed whether a commercially available antibody against human NGF can be applicable for the detection of the protein.

* Corresponding author at: Hiroshi Matsuda, Laboratory of Veterinary Molecular Pathology and Therapeutics, Division of Animal Life Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan.
E-mail address: hiro@cc.tuat.ac.jp (H. Matsuda).
[1] Yosuke Amagai, Hiroaki Sato, and Saori Ishizaka equally contributed this work.

0737-0806/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2016.06.001

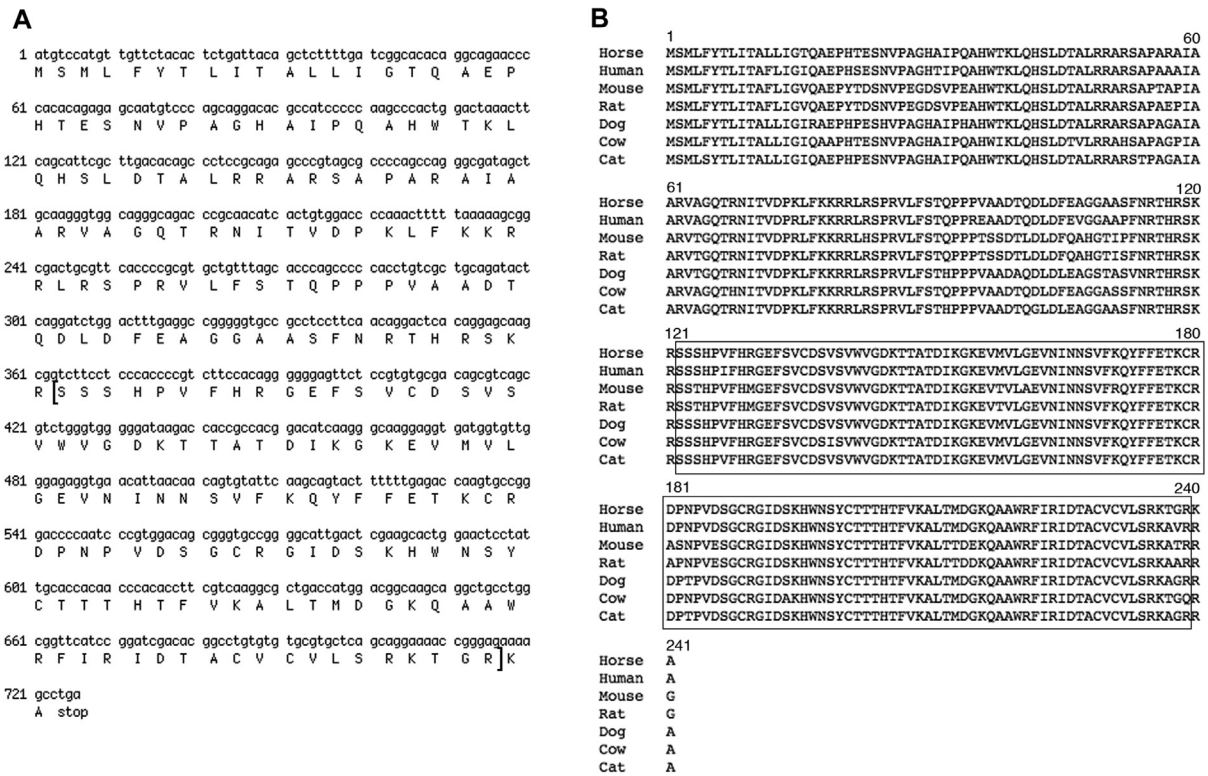

Fig. 1. (A) Sequence of cloned NGF sequence. Square brackets indicate the beginning and the end of the predicted β-NGF sequence based on the amino acid comparison between species. (B) Comparison of amino acids of prepro-NGF in species. Framed sequences are corresponded to the β-NGF in each species. β-NGF, β-nerve growth factor; NGF, nerve growth factor.

2. Materials and Methods

2.1. Animals

All experiments with horses had ethical permission along with the standards specified in the guidelines of the University Animal Care and Use Committee of the Tokyo University of Agriculture and Technology, as well as with the guidelines for the use of laboratory animals provided by the Science Council of Japan. A total of 10 healthy male adults horses (seven Thoroughbreds and three warmblood horses) were included. Approximately 30 mL of blood were obtained from one Vena jugularis externa of each horse into conical tubes containing EDTA (Maxim Biotech Inc, San Francisco, CA). After hemolysis with ACK lysis buffer (0.15 M NH_4Cl, 1.0 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.2), total RNA from white blood cells was extracted with ISOGEN (Nippongene, Tokyo, Japan) and reverse transcribed into cDNA by using random oligo(dT) primers and PrimeScript (Takara Bio, Shiga, Japan) according to the manufacturer's instructions.

2.2. Cloning of Equine NGF

Polymerase chain reactions (PCRs) with PrimeStar Max DNA polymerase (Takara Bio) were carried out using a pair of primers 5′-ATGTCCATGTTGTTCTACACT-3′ (sense primer) and 5′-TCAGGCTTTTCTCCCGGTTTT-3′ (antisense primer) and the equine blood cDNA as templates. Polymerase chain reaction products were cloned into a pGEM-T TA cloning vector (Promega, Madison, WI), followed by PCR reactions using BigDye Terminator v3.1 Cycle Sequencing kits (Life Technologies, Gaithersburg, MD) with M13-forward and M13-reverse primers. The sequence was analyzed by ABI3100 sequencer (Life Technologies) according to the manufacturer's instruction.

2.3. Cell Culture

CHO-K1 cells were obtained from Riken Cell Bank (Tsukuba, Japan). Cells were cultured in RPMI 1640 medium (Life Technologies) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/mL penicillin, and 100 μg/mL streptomycin in a humid atmosphere of 5% CO_2 in air at 37°C.

2.4. Expression of Equine NGF and Immunocytochemistry

The β-nerve growth factor (β-NGF) sequence was cloned into pIRES2-AcGFP vector (Clontech, Palo Alto, CA), which is designated pIRES2-β-NGF-AcGFP and transfected into CHO-K1 cells by using a Cell Line Nucleofector kit T (Lonza, Walkersville, MD) with a Nucleofector I electroporation device (Lonza) according to the manufacturer's instructions. Cells were cultured for 48 hours in the culture medium and then fixed with 4% paraformaldehyde (Wako,