Neuroscience Letters 501 (2011) 179–184

Contents lists available at ScienceDirect

Neuroscience Letters

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

Partial rescue of NT-3 null mutant phenotype by a PDGF-β regulated transgene

Jarosław J. Barski[a,b,*], Christian Helbig[c], Michael Meyer[d]

[a] Center for Experimental Medicine, Medical University of Silesia, ul. Medyków 4, 40-752 Katowice, Poland
[b] Department of Physiology, Medical University of Silesia, ul. Medyków 18, 40-752 Katowice, Poland
[c] Formerly Max-Planck Institute of Psychiatry, Am Klopferspitz 18A, Martinsried, Germany
[d] Institute of Physiology, Ludwig-Maximilians-University, Pettenkoferstrasse 12, 80336 Munich, Germany

ARTICLE INFO

Article history:
Received 25 March 2011
Received in revised form 6 June 2011
Accepted 10 June 2011

Keywords:
NT-3
Dorsal root ganglion
Muscle spindles
Sensory ataxia
Rescue

ABSTRACT

The phenotype of neurotrophin-3 (NT-3) null mutant mice is characterized by sensory ataxia and early postnatal death. Previous analysis revealed a severe depletion of peripheral sensory, sympathetic and parasympathetic neurons. Most of the deficits are established early during embryonic development. Whereas absence of proprioceptive afferents can explain the sensory ataxia, the reasons for early postnatal death are unclear. To circumvent the limitations imposed by early mortality of null mutants we generated mouse line expressing NT-3 transgenes driven by the platelet-derived growth factor β-chain (PDGF-β) promoter, which is known to be active in neurons and mesenchyme derivatives. Mice carrying one or two *PDGF-NT3* transgenes on a background null for wildtype NT-3 were generated by crossing with an NT-3 null strain. Although still ataxic, mice from this cross could survive for periods longer than a year. Histological analysis revealed a limited rescue of muscle spindles and parvalbumin immunoreactive sensory neurons.

Neurotrophin-3 (NT-3) belongs to the family of related growth factors including the nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-4/5, and NT-6 [19]. Animals that are null mutants for NT-3 display severe ataxia and die within the first postnatal week [1,2,13]. Analysis of mice lacking NT-3 demonstrates the absence of main peripheral proprioceptive sensory organs: muscle spindles and Golgi tendon organs [12,16]. In lumbar dorsal root ganglia (DRG) about 50% of neurons are lost, and detailed analysis reveals that almost all classes of DRG neurons are affected by the lack of NT-3 [1]. The most dramatic effect however is visible in the population of parvalbumin- and carbonic anhydrase-positive sensory neurons [17]. These proteins are markers for neurons innervating muscle spindles by Ia-type fibers and Golgi tendon organs by II-type fibers. Both neuronal populations are almost completely absent in lumbar DRGs together with their central and peripheral projections. The absence of NT-3 is also responsible for the complete ablation of motor innervation of muscle spindles by γ motoneurons [16,17]. Strong reduction of sensory neurons is visible in the cochlear ganglion (~85%), trigeminal ganglion (~60%) and petroso-nodosal complex (NOD) (~30%) [9,10,13,35]. In addition the lack of NT-3 results in severe (~50%) reduction in number of superior cervical ganglion (SCG) neurons [10,39]. Investigations aiming at understanding the lethal phenotype of NT-3 mutants discovered impairments in the cardiovascular system showing the existence of non-neuronal targets for NT-3 [8,32]. Because of the complexity of NT-3 null mutant phenotype and early lethality the reason(s) of early death remains unclear. To circumvent these limitations we generated a mouse line expressing a NT-3 transgene driven by the platelet-derived growth factor β-chain (PDGF-β) promoter and crossed them with heterozygous mice for an NT-3 null-mutant strain. The PDGF-B chain is known to be expressed in a variety of neuronal subpopulations [28,29], its promoter drives transgene expression in similar populations and derivatives of the mesenchyme [21,29]. It thus appeared to be suitable for our experiments aiming at elucidation of the developmental significance of different sources of NT-3.

The generation of NT-3 null mutant mice has been described previously [2]. PDGFNT-3 line was generated as follows: the expression vector pSKPINT-3 was constructed on the backbone of pBluescript SK^+ (Stratagene). The PDGF-β promoter was isolated from the plasmid pSISCAT6a [28]. β-globin intron and SV40 polyadenylation signal were isolated from plasmid pSG5 (Stratagene) (Fig. 1A). Vector devoid of prokaryotic sequences was injected into the male NMRI pronucleus. Heterozygous founder animals were crossed with NMRI wild type animals and F1 heterozygotes were mated to establish the PDGFNT-3 line.

Mice heterozygous for the inactive NT-3 allele were mated with PDGFNT-3 animals and double heterozygotes were mated to generate the rescue line.

* Corresponding author at: Center for Experimental Medicine, Medical University of Silesia, ul.Medyków 4, 40-752 Katowice, Poland. Tel.: +48 32 208 8593.
E-mail address: jbarski-cmd@sum.edu.pl (J.J. Barski).

0304-3940/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.06.063

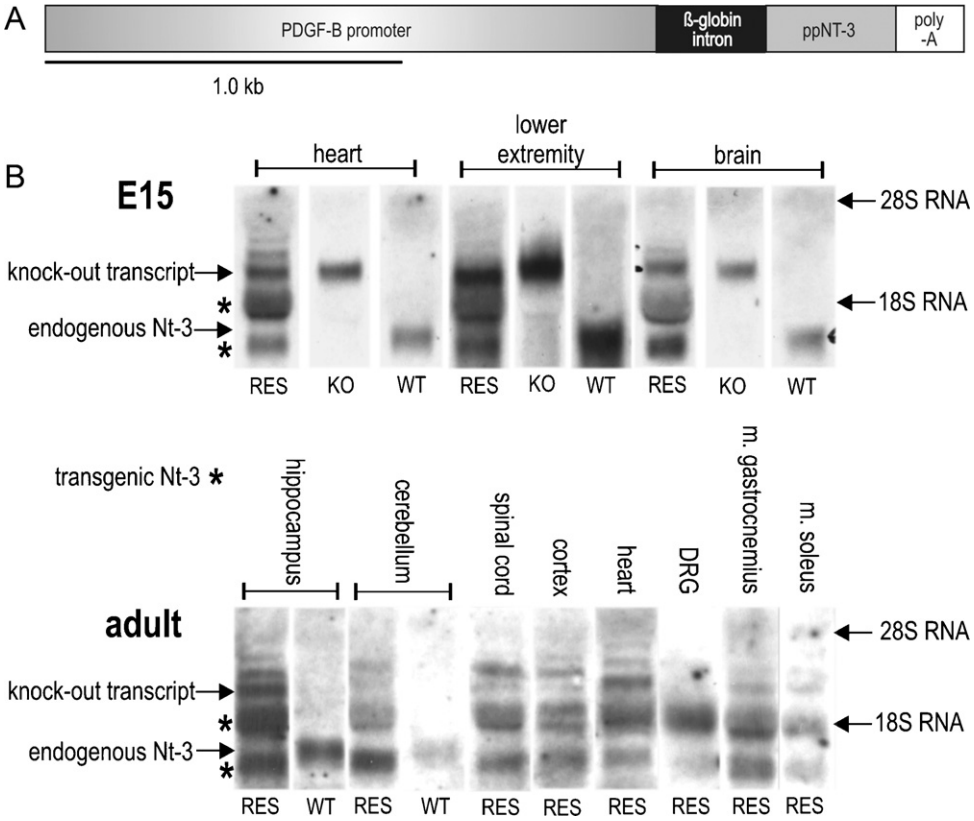

Fig. 1. (A) Schematic diagram PDGFNT-3 vector. ppNT-3–NT-3 cDNA. (B) Northern blot analysis of rescue line. RES–NT-3$^{-/-}$/*PDGFNT-3*/*PDGFNT-3* animals, KO–NT-3$^{-/-}$ animals, WT–NT-3$^{+/+}$ animals.

RNA isolation was described elsewhere [5]. 10 μg of RNA were glyoxylated and separated on 1.4% agarose gels, blotted onto nylon membranes (Amersham, Germany), and immobilized by UV-cross linking. An antisense riboprobe was transcribed from the cDNA of mouse NT-3 (nucleotide 308–777; GenBank accession no. NM_008742) in the presence of digoxigenin-11-uridine-5′-triphosphate (Boehringer, Germany). Hybridization and detection was essentially done as described [22].

P1 animals were anaesthetized with chloral hydrate and perfused transcardially with PBS and 4% paraformaldehyde. Whole animals were postfixed and cryopreserved in 30% sucrose. Transverse 20 μm thick frozen sections were cut from the lumbar body part for DRG analysis and transverse sections of the head were used for SCG or NOD. For total neuronal counts sections were stained with cresyl violet. To estimate the number of parvalbumin positive neurons and muscle spindles, sections were stained immunohistochemically with rabbit anti-parvalbumin antibody 1:400 (SWant, Switzerland) and DTAF-coupled anti-rabbit antibody 1:100 (Jackson ImmunoResearch Laboratories, USA). Cresyl violet stained sections were analyzed in a bright-field microscope (Axiophot ZEISS, Germany). Fluorescence was analyzed using a confocal microscope (Leitz, Germany). For statistical analysis one-way ANOVA was used.

To analyze the heart structure whole hearts were dissected from the chest, dehydrated and embedded in paraffin. Serial sections were stained with haematoxylin/eosin and analyzed by light microscopy.

Mouse null mutants for NT-3 (NT-3$^{-/-}$) generated in our laboratory were phenotypically similar to NT-3 mutants generated by other groups and all died within the first postnatal week, displaying strong ataxia [1,2]. NT-3$^{-/-}$ animals expressing one or two alleles of the transgene (NT-3$^{-/-}$/*PDGFNT-3*/+; NT-3$^{-/-}$/*PDGFNT-3*/*PDGFNT-3*) were analyzed for transgene expression and possible rescue effects. NT-3 null mutants (NT-3$^{-/-}$), wild type animals (NT-3$^{+/+}$), animals expressing endogenous NT-3 and one or two alleles of transgenic NT-3 (NT-3$^{+/+}$/*PDGFNT-3*/+; NT-3$^{+/+}$/*PDGFNT-3*/*PDGFNT-3*) were used for comparison. Animals from the primary NT-3 knockout line served as an additional control group.

Since E15 is the starting point of PDGF-β expression, we looked first at transgene expression in E15 embryos (Fig. 1B). In lanes with RNA from NT-3$^{+/+}$ animals (WT), only a single band is visible. It represents the product of the wild type locus. A single band is also visible in lanes with RNA from NT-3$^{-/-}$ animals (KO). This band however is longer, because the coding exon is transcribed together with the inserted *neo* cassette. RNA from rescue animals (RES) is represented by three bands (Fig. 1B). The longest one comes from the knockout transcript. RNA transcribed from the PDGF-β transgene is represented by two bands, presumably because of alternative splicing. The strongest signal was visible in RNA samples extracted from lower extremity and slightly weaker signals in extracts from heart and brain. More detailed analysis was done on RNA from adult animals. Bands specific for transgenic NT-3 were visible in all analyzed parts of CNS, but the most intensive signal was detected in hippocampal extracts. Strong expression was also detected in heart, muscles, and DRGs.

In transverse spinal cord (SC) sections of NT-3$^{+/+}$ animals a full set of parvalbumin-positive innervation is visible (Fig. 2A). Parvalbumin-positive fibers are present in dorsal and ventral roots (arrowheads), projecting also dorso-ventrally (dashed line with arrowheads) towards motor neurons (dashed eclipse). Analysis of SC sections of NT-3$^{-/-}$ animals lacked parvalbumin reactivity (Fig. 2B). The same applies for SC of NT-3 null mutants expressing one copy of *PDGFNT-3* transgene (NT-3$^{-/-}$/*PDGFNT-3*/+) (data not shown). Parvalbumin-positive fibers can however be observed in