ELSEVIER

Neuroscience Letters 301 (2001) 107–110

Neuroscience Letters

www.elsevier.com/locate/neulet

The expression profiles of neurotrophins and their receptors in rat and chicken tissues during development

Fanny C.F. Ip, Janet Cheung, Nancy Y. Ip*

Department of Biochemistry and Biotechnology Research Institute, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, PR China

Received 26 January 2001; received in revised form 2 February 2001; accepted 5 February 2001

Abstract

Neurotrophic factors are target-derived proteins that promote the survival and differentiation of the innervating neurons. Increasing evidence indicate the involvement of these factors and receptors during the formation and maturation of the neuromuscular junction. To gain further insight on the expression pattern of these factors and receptors in developing spinal cord and skeletal muscle during the critical stages of synapse formation, a systematic study was performed with chicken and rat tissues using Northern blot analysis. The expression of all the neurotrophins was detected in skeletal muscle early in development, coincidental with the appearance of their corresponding receptors in the spinal cord. Taken together, the similar regulatory patterns observed in both rat and chicken tissues suggest that the potential roles of neurotrophins at the neuromuscular synapse are conserved throughout evolution.

Keywords: Synaptogenesis; Neuromuscular junction; Trophic factors; Neurotrophins; Trk receptors; Development

Neurotrophic factors are proteins that are often synthesized by target tissues to promote the survival and differentiation of the innervating neurons. One major class of neurotrophic factors is collectively known as the neurotrophins (for reviews see Refs. [10,11]). Nerve growth factor (NGF) is the proto-type of the neurotrophin family with other members including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), neurotrophin-6 (NT-6), and neurotrophin-7 (NT-7). NT-6 and NT-7 are neurotrophins that have only been reported in fish and their mammalian counterparts have not yet been identified [6,12]. The neurotrophins bind and activate a family of receptor tyrosine kinase known as Trks. There are three members in this family, TrkA, TrkB and TrkC, which exhibit ligand specificity for different neurotrophins. NGF, NT-6 and NT-7 are the preferred ligands for TrkA, BDNF and NT-4/5 activate TrkB, NT-3 activates TrkC although it also binds TrkB in some cells [12,18]. The expression of specific Trk receptors in cells determines the responsiveness to the corresponding neurotrophins (for reviews see Refs. [9,10]).

Besides the important roles for the neurotrophic factors in the structural maintenance, synaptic plasticity, as well as the repair of the central nervous system (for reviews see Refs. [9–11]), increasing evidence indicates the critical involvement of these factors and receptors in the formation and maturation of a peripheral synapse, the neuromuscular junction. The formation of neuromuscular junction involves reciprocal interactions of molecules derived from both the presynaptic motor neuron and postsynaptic muscle membrane during the events of innervation and synapse elimination [1]. Agrin and neuregulin, are the two major nerve-derived factors that regulate most of the important events during the course of synaptogenesis [2,16]. While agrin regulates the precise localization of a variety of postsynaptic proteins, neuregulin specifically induces the synapse specific gene transcription. The hallmark example is the protein clustering and the specific subunit transcription of acetylcholine receptor on the subsynaptic muscle membrane. On the other hand, muscle-derived factors, such as the neurotrophic factors, have been shown to influence the sprouting and differentiation of the presynaptic nerve terminals. For example, NT-3 and BDNF have been shown to potentiate the synaptic activity of developing neuromuscular synapses in cultures [7,14], while NT-4/5 induces sprouting of the intact adult motor neuron [4].

* Corresponding author. Tel.: +852-2358-7304; fax: +852-2358-2765.

E-mail address: boip@ust.hk (N.Y. Ip).

0304-3940/01/$ - see front matter
PII: S0304-3940(01)01603-2

More recently, neurotrophins have been found to directly regulate agrin-induced postsynaptic differentiation and increase neuregulin expression [13,20]. In particular, muscle-derived NT-3 has been demonstrated to increase acetylcholine receptor clusters in a neuron-muscle co-culture system [3]. Disruption of TrkB mediated signaling results in the disassembly of agrin-induced acetylcholine receptor clusters at the postsynaptic muscle membrane [5]. All these findings demonstrate the potential functions that may be elicited by the neurotrophic factors at the neuromuscular synapse in vivo.

To support the potential role of neurotrophins implicated in neuromuscular junction formation, it is important to delineate the expression profiles of these factors and receptors in developing spinal cord and skeletal muscle during the critical stages of synapse formation, i.e. embryonic day 7 (E7) to E10 in chick and E14–E16 in rat [15]. Although scattered information regarding the expression of these factors or their receptors during development has been reported, a systematic analysis in both rat and chicken tissues is lacking. The present study aims to systematically examine the spatial and temporal expression profiles of the neurotrophins and Trk receptors during development. Our findings reveal striking parallels in the expression patterns of these factors and receptors in both rat and chicken tissues, and lend support to the potential functions of these factors at the neuromuscular synapse.

After the animals were sacrificed by decapitation or carbon dioxide inhalation, different tissues (brain, spinal cord and skeletal muscle) of chicken (New Hampshire) and rat (Sprague–Dawley) were dissected from various developmental stages ranging from early embryonic stages, postnatal stages to adult. The dissected tissues were then immediately frozen by liquid nitrogen and stored at −80° C before use. Total RNA extraction and Northern blot analysis were performed as previously described [8]. Briefly, brain and skeletal muscle were homogenized in lithium chloride/urea solution, while spinal cord was extracted in guanidium thiocyanate. RNA samples were subjected to formaldehyde agarose gel electrophoresis and visualized under UV-illumination by ethidium bromide staining. Full-length cDNAs of all factors and receptors were used as template, except for chicken trkA (flanking 473–1580 bp of coding region). Probes were randomly labeled with [α^{-32}P]dCTP using Megaprime labeling kit (Amersham-Pharmacia Biotech, UK).

The expression of NGF was detected in most of the chicken and rat tissues examined (Fig. 1A,C) with the transcript size of ~1.4 kb. Tissue specific regulation of NGF transcript was observed. For example, a gradual increase was observed in developing chicken brain and spinal cord. On the other hand, NGF transcript was high in both chicken and rat muscle during embryonic stages (E7–E18) and down-regulated to a very low level after birth (Fig. 1A,C). TrkA, the cognate receptor for NGF, was detected with a transcript size of ~3 kb in both chicken and rat tissues (Fig. 1B,D). While the mRNA expression of TrkA increased in chick cerebrum and rat brain during development, high level of transcript was already found in E7 chick cerebellum followed by a dramatic decrease at E12 (Fig. 1B,D). More importantly, TrkA transcript was detected in spinal cord, coincidental with the expression of NGF in muscle (Fig. 1). Interestingly, TrkA transcript was also detected during the early embryonic muscle of both species.

Two transcripts of BDNF could be detected in both chicken (~4.5 and ~1.4 kb) and rat (~4.0 and ~1.6 kb) (Fig. 2A,C). The regulatory pattern was very similar in all tissues examined (Fig. 2A,C). While BDNF mRNA was up-regulated to an abundant level in chicken and rat brain throughout the postnatal stages to adult, low level of transcripts was found in spinal cord and muscle during the early embryonic stages and decreased to an undetectable level after birth (Fig. 2A,C). On the other hand, multiple transcripts of NT-4/5 (~9, ~4, ~2.1 and ~1.1 kb) were observed (Fig. 2D). While a constitutive level of transcripts was detected in rat brain, the expression of NT-4/5 different transcripts was differentially regulated in muscle during development (Fig. 2D). An increase of TrkB transcripts

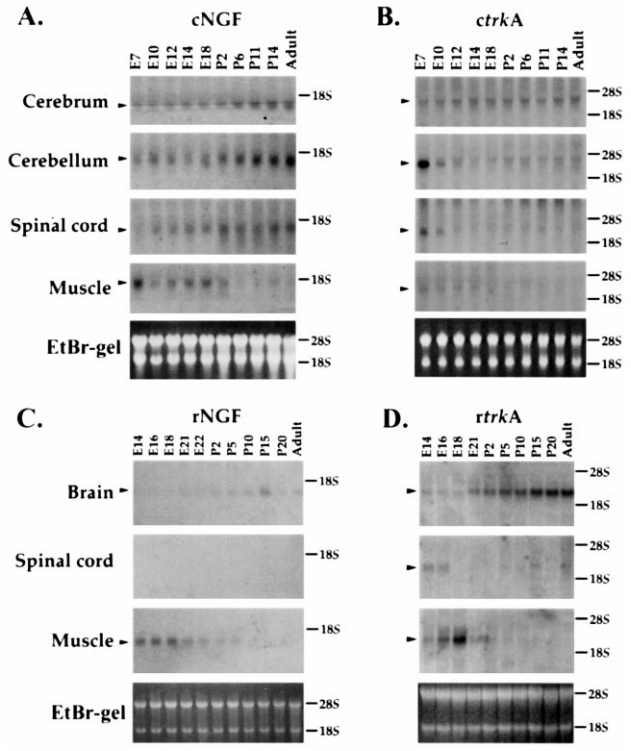

Fig. 1. Developmental expression of NGF and TrkA receptor in chicken and rat tissues. The expression of these transcripts was examined in brain, spinal cord and muscle of chicken (A,B) and rat (C,D). Fifteen micrograms of total RNA were loaded; the ribosomal RNA bands (18*S* and 28*S*) are indicated on the right. Detectable transcripts are indicated by arrowheads. Ethidium bromide stained gels (EtBr-gel) of skeletal muscle and spinal cord RNAs are shown at the bottom panels of NGF and TrkA, respectively.

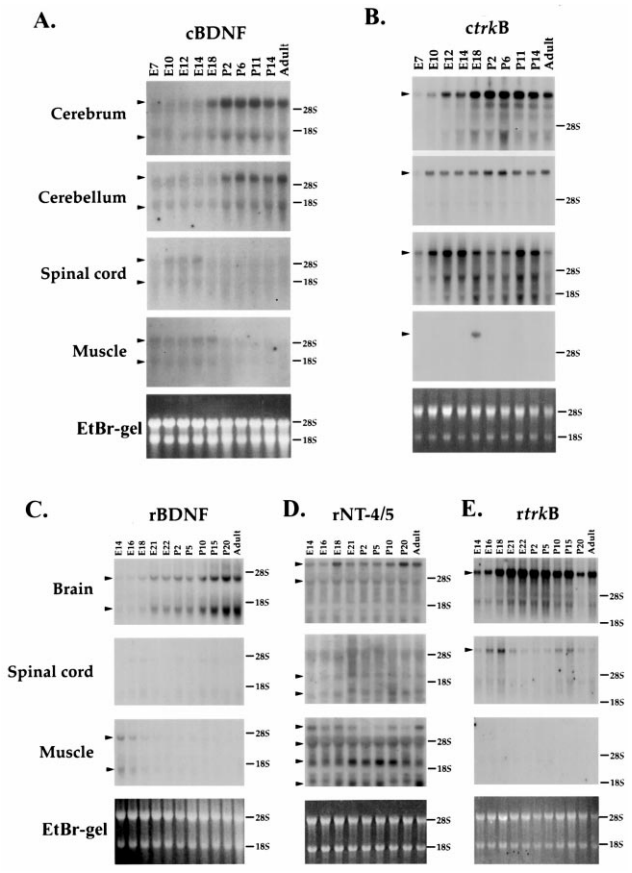

Fig. 2. Developmental expression of BDNF, NT-4/5 and trkB. The expression of these transcripts was examined in brain, spinal cord and muscle of chicken (A,B) and rat (C,D). Fifteen micrograms of total RNA were loaded; the ribosomal RNA bands (18*S* and 28*S*) are indicated on the right. Detectable transcripts are indicated by arrowheads. Ethidium bromide stained gels (EtBr-gel) of skeletal muscle and spinal cord RNAs are shown at the bottom panels of BDNF or NT-4/5 and TrkB, respectively.

(~9.0 kb) was observed in brain and spinal cord of E7–E10 chick and E14–E18 rat, and remained at detectable levels throughout the development (Fig. 2B,E).

The chicken NT-3 transcripts (~4 and ~1.3 kb) were observed in all tissues examined (Fig. 3A). A relatively constant level of both NT-3 transcripts was found in cerebrum, cerebellum, and muscle during development with a barely detectable level in chicken spinal cord. A single transcript (~1.4 kb) of rat NT-3 was observed in all types of tissues examined (Fig. 3C). The mRNA expression of NT-3 in rat brain increased dramatically from E18 to E21, down-regulated at P10 and remained at low level in adult (Fig. 3C). Low level of NT-3 mRNA was detected in spinal cord during the embryonic stages and decreased after birth. Similarly, abundant level of NT-3 transcript was found in rat muscle of E14–E18 and showed a ~20-fold decrease in adulthood (Fig. 3C). Multiple transcripts of chicken TrkC mRNA could be detected (~6.3 and ~10 kb), similar to that of rat (~5.5 and ~14 kb; Fig. 3B,D). The mRNA expression of TrkC showed a gradual increase pattern in brain of both species during development. In chicken spinal cord, prominent expression of TrkC transcripts was detected from E7 to E12, followed by a decrease and remained high level in adult chicken (Fig. 3B,D). In chicken muscle, TrkC mRNA was only found during embryonic stages and decreased after birth.

A functional role for BDNF and NT-3, but not NGF, have been implicated during the formation of neuromuscular junction (NMJ) [13,14,19]. In the present study, the expression of all the neurotrophins, including NGF, was detected in skeletal muscle during the critical stages of synapse formation, coincidental with the expression of their corresponding receptors in the spinal cord. Our findings in the present study demonstrate that these regulatory patterns are

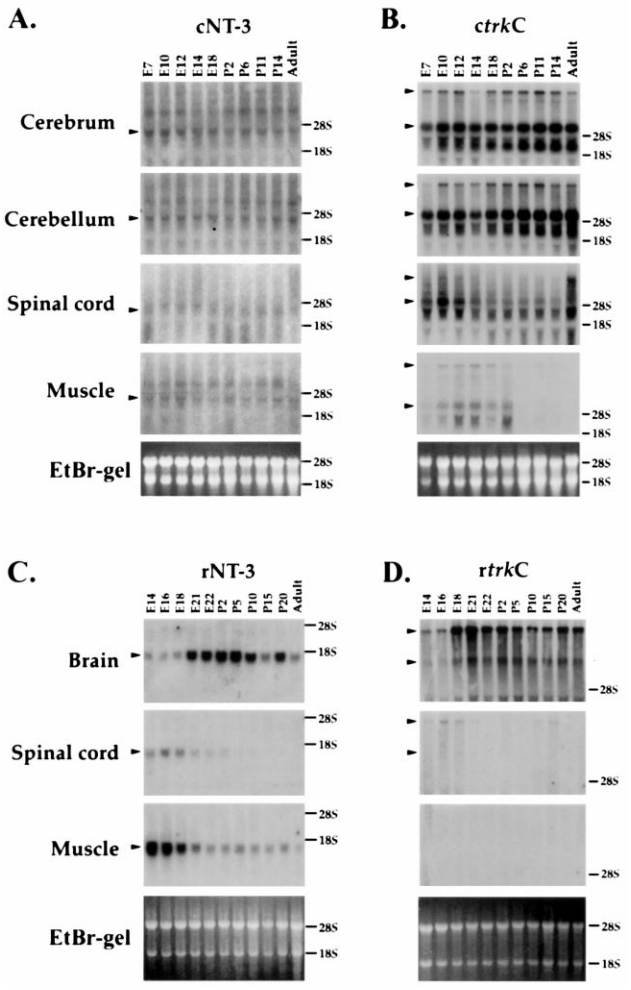

Fig. 3. Developmental expression of NT-3 and trkC. The expression of these transcripts was examined in brain, spinal cord and muscle of chicken (A,B) and rat (C,D). Fifteen micrograms of total RNA were loaded; the ribosomal RNA bands (18*S* and 28*S*) are indicated on the right. Detectable transcripts are indicated by arrowheads. Ethidium bromide stained gels (EtBr-gel) of skeletal muscle and spinal cord RNAs are shown at the bottom panels of NT-3 and TrkC, respectively.

strikingly similar between rat and chicken, suggesting that the involvement of the neurotrophins at the neuromuscular synapse is conserved throughout evolution. Although the neurotrophins show an overlapping developmental expression profile, they might act to influence different components of the NMJ formation, reminiscent of their distinct properties on different populations of neurons during development. Unlike BDNF and NT-3, NGF has not previously been suggested to be involved in the NMJ formation. However, a recent study has reported that NGF selectively regulates agrin mRNA induction and alternative splicing in PC12 cells by Ras-dependent pathway [17]. Together with our finding on the regulatory expression profile of NGF and trkA in muscle and spinal cord, it is possible that NGF may play a role in NMJ formation by regulating agrin expression during those critical stages of synapse formation. In addition, the transient co-expression of NGF and its cognate receptor during embryonic stages of chicken and rat muscle suggests a novel function for NGF during that critical period, possibly in an autocrine or paracrine manner.

We thank Dr Karl Tsim for helpful discussion throughout the course of this study. The study was supported by the Research Grants Council of Hong Kong (HKUST 568/95M).

[1] Bennett, M.R., Synapse formation molecules in muscle and autonomic ganglia: the dual constraint hypothesis, Prog. Neurobiol., 57 (1999) 225–287.
[2] Fischbach, G.D. and Rosen, K.M., ARIA: a neuromuscular junction neuregulin, Annu. Rev. Neurosci., 20 (1997) 429–458.
[3] Fu, A.K.Y., Ip, F.C.F., Lai, K.O., Tsim, K.W.K. and Ip, N.Y., Muscle-derived neurotrophin-3 increases the aggregation of acetylcholine receptors in neuron-muscle co-cultures, NeuroReport, 8 (1997) 3895–3900.
[4] Funakoshi, H., Belluardo, N., Arenas, E., Yamamoto, Y., Casabona, A., Persson, H. and Ibáñez, C.F., Muscle-derived neurotrophin-4 as an activity-dependent trophic signal for adult motor neuron, Science, 268 (1995) 1495–1499.
[5] Gonzalez, M., Ruggiero, F.P., Chang, Q., Shi, Y.J., Rich, M.M., Kraner, S. and Balice-Gordon, R.J., Disruption of Trkb-mediated signaling induces disassembly of postsynaptic receptor clusters at neuromuscular junctions, Neuron, 24 (1999) 567–583.
[6] Götz, R., Koster, R., Winkler, C., Raulf, F., Lottspeich, F., Schartl, M. and Thoenen, H., Neurotrophin-6 is a new member of the nerve growth factor family, Nature, 372 (1994) 266–269.
[7] He, X., Yang, F., Xie, Z. and Lu, B., Intracellular Ca(2+) and Ca(2+)/calmodulin-dependent kinase II mediate acute potentiation of neurotransmitter release by neurotrophin-3, J. Cell. Biol., 149 (2000) 783–792.
[8] Ip, F.C.F., Fu, A.K.Y., Tsim, K.W.K. and Ip, N.Y., Cloning of the alpha component of the chick ciliary neurotrophic factor receptor: developmental expression and down-regulation in denervated skeletal muscle, J. Neurochem., 65 (1995) 2393–2400.
[9] Ip, N.Y. and Yancopoulos, G.D., Neurotrophic factor receptors: just like other growth factor and cytokine receptors? Curr. Opin. Neurobiol., 4 (1994) 400–405.
[10] Ip, N.Y. and Yancopoulos, G.D., The neurotrophins and CNTF: two families of collaborative neurotrophic factors, Annu. Rev. Neurosci., 19 (1996) 491–515.
[11] Kaplan, D.R. and Miller, F.D., Neurotrophin signal transduction in the nervous system, Curr. Opin. Neurobiol., 10 (2000) 381–391.
[12] Lai, K.O., Fu, W.Y., Ip, F.C.F. and Ip, N.Y., Cloning and expression of a novel neurotrophin, NT-7, from Carp, Mol. Cell. Neurosci., 11 (1998) 64–76.
[13] Loeb, J.A. and Fischbach, G.D., Neurotrophic factors increase neuregulin expression in embryonic ventral spinal cord neurons, J. Neurosci., 17 (1997) 1416–1424.
[14] Lohof, A.M., Ip, N.Y. and Poo, M.M., Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF, Nature, 363 (1993) 350–353.
[15] Moody-Corbett, F., Formation of the vertebrate neuromuscular junction, Dev Biol (NY 1985), 2 (1986) 605–635.
[16] Ruegg, M.A. and Bixby, J.L., Agrin orchestrates synaptic differentiation at the vertebrate neuromuscular junction, Trends Neurosci., 21 (1998) 22–27.
[17] Smith, M.A., Fanger, G.R., O'Connor, L.T., Bridle, P. and Maue, R.A., Selective regulation of agrin mRNA induction and alternative splicing in PC12 cells by Ras-dependent actions of nerve growth factor, J. Biol. Chem., 272 (1997) 15675–15681.
[18] Snider, W.D., Functions of the neurotrophins during nervous system development: what the knockouts are teaching us, Cell, 77 (1994) 627–638.
[19] Stoop, R. and Poo, M.-M., Synaptic modulation by neurotrophic factors: differential and synergistic effects of brain-derived neurotrophic factor and ciliary neurotrophic factor, J. Neurosci., 16 (1996) 3256–3264.
[20] Wells, D.G., McKechnie, B.A., Kellar, S. and Fallon, J.R., Neurotrophins regulate agrin-induced postsynaptic differentiation, Proc. Natl. Acad. Sci. USA, 96 (1999) 1112–1117.