C. R. Acad. Sci. Paris, Sciences de la vie/Life sciences, 1993; 316 : 1161-3

Towards a comprehensive understanding of the trophic support of motoneurons

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Reprints : H. Thoenen

ABSTRACT

Motoneurons played an essential role in establishing the concept of target-mediated support of innervating neurons. However, it took several decades until molecules were identified which trophically support motoneurons *in vitro* and *in vivo*. The most potent molecule identified so far is ciliary neurotrophic factor (CNTF). It is expressed as a cytosolic molecule in myelinating Schwann cells rather than in skeletal muscle in the postnatal period and therefore does not qualify as a target-derived neurotrophic factor regulating motoneuron survival during embryonic development. However, the inactivation of CNTF by gene targeting experiments results in progressive atrophy and degeneration of motoneurons, demonstrating that CNTF plays an essential role as a maintenance factor for motoneurons postnatally. Secretory molecules which are expressed in skeletal muscle during embryonic development and which support motoneurons in culture and partially also *in vivo* include members of the NGF gene family (BDNF, NT-3, NT-4/5), FGF-5, IGF-I, and LIF. The evaluation of the physiological importance of these molecules is under investigation. ▲

Key words : neurotrophic molecules, CNTF, gene targeting, NGF gene family, FGF-5, LIF, IGF-I.

When experimental embryologists, in the first part of this century, established the concept of the trophic support of innervating neurons by target tissues, spinal motoneurons played an essential role (see [1, 2]). However, for a long time, little progress was made in identifying and characterizing the molecules responsible for this trophic support. The situation has changed dramatically within the last few years in that it has been demonstrated that the trophic support of motoneurons is not only restricted to the target tissues in the proper sense, *i. e.* skeletal muscle, but that spinal motoneurons are also trophically supported by glial cells ensheathing the axons of motoneurons [3-5]. The development of appropriate culture systems for (pure) motoneurons played an essential role in accomplishing this rapid progress [6-8]. However, there are also limitations to the conclusiveness of the results obtained in a given culture system with respect to the physiological situation (discussed extensively in [5]).

In the following, we will summarize the present state of knowledge of the physiological and pathophysiological role of ciliary neurotrophic factor (CNTF), the most potent trophic molecule for motoneurons identified so far. Although CNTF does not satisfy the criteria of a target-derived neurotrophic factor for motoneurons (see [3, 5]), it nevertheless plays an essential role in the trophic support of motoneurons in the postnatal period [4] and, most likely also, under pathophysiological conditions as a "lesion factor" [9, 10]. Then we will briefly describe the most essential candidates for a target-derived neurotrophic support of motoneurons which have very recently been identified and which would fulfill the requirements for the regulation of the selective motoneuron survival during a critical period of the embryonic development and which could also function as maintenance factors in the postnatal period (summarized in [5]).

Ciliary neurotrophic factor (CNTF)

CNTF was initially identified and partially purified from chick eye extracts in the search for a target-derived neurotrophic factor for innervating parasympathetic ciliary neurons [11, 12]. Very soon it became apparent that the spectrum of actions exhibited by CNTF under *in vitro* conditions is much broader and includes effects on a variety of peripheral and central neurons as well as glial cells (summarized in [3]). In highly enriched cultures of embryonic day 6 chick motoneurons, CNTF proved to be, by far, the most potent neurotrophic molecule exhibiting a maximal survival effect of over 60 %, the half-maximal survival effect being reached at a concentration of 30 pg/ml, followed by basic fibroblast growth factor (bFGF) with a maximal survival effect of 50 % showing half-maximal survival activity at a concentration of 280 pg/ml [6].

The elucidation of the primary structure of CNTF by molecular cloning [13, 15] indicated that CNTF is a cytosolic molecule, lacking the characteristic leader sequence of ER-Golgi secreted molecules [13, 15]. These structural features are in agreement with the observation that both in primary cultures of astrocytes and Schwann cells (which both synthesize CNTF), and transfected Hela and Cos cell the quantity of CNTF in the culture

medium, in comparison to the cell lysate, only represents a minute proportion, compatible with the assumption that this small quantity could exclusively result from normal cell death under the given culture conditions. However, these results do not exclude the possibility that a very small proportion of CNTF is released in the culture medium by an unconventional regulated pathway, as postulated for bFGF [14]. Moreover, it then also became apparent that CNTF is only expressed in the postnatal period by myelinating Schwann cells and a subpopulation of type-I astrocytes, precluding a function as a target-derived (skeletal muscle) molecule which regulates the survival of motoneurons during the period of naturally occurring cell death between embryonic day 15 and birth in rats (see [3, 5, 8, 10]). The period of physiological motoneuron cell death is followed by a period of high sensitivity to axotomy, *i. e.* transection of the axons of motoneurons in newborn animals results in almost complete degeneration of the corresponding cell bodies [9, 16]. This high vulnerability to axonal lesion of motoneurons decreases in the following 3 to 4 postnatal weeks, the time course showing a striking reciprocity to the increase of CNTF in the myelinating Schwann cells of the sciatic nerve. This led us to propose the hypothesis that CNTF could act as a "lesion factor" for motoneurons. Indeed, the local administration of CNTF to the transected facial nerve of newborn rats virtually completely prevented the otherwise occurring degeneration of motoneurons (9]. In subsequent experiments in which the sciatic nerve of adult rats was lesioned, it was demonstrated that, although CNTF mRNA was strongly reduced in the peripheral part of the nerve undergoing Wallerian degeneration [10], substantial quantities of biologically active CNTF protein were still available, exceeding the biological activity of NGF (synthesized by non-neuronal cells in the lesioned sciatic) by a factor of about 1 000 [10]. This supported the interpretation that CNTF becoming available after nerve lesion could represent the first emergency factor to rescue motoneurons which is then followed by the gradually increasing synthesis of BDNF by Schwann cells [17]. The latter factor could then take over the function of CNTF and also promote the regeneration of motoneuron axons in the periphery toward their target, *i. e.* the skeletal muscle.

Although the developmental time course of expression and the cellular localization of CNTF excludes a function of CNTF as a target-derived neurotrophic molecule, the question arose as to whether CNTF could play a physiological role in trophically supporting motoneurons in the postnatal phase. It could be predicted that only a minute proportion of the cytosolic CNTF need become available to the axons of motoneurons either by a non-conventional regulated release or by discontinuities in the cell membranes of the CNTF-producing Schwann cells resulting, *e. g.*, from repetitive microtrauma. The use of the long-term administration of CNTF antibodies as a means to evaluate a potential physiological function of CNTF promised little return, in that the antibodies were not expected to reach the site of contact between the motoneuron axons and the ensheahing Schwann cells. The inactivation of the CNTF gene by homologous recombination thus was the method of choice [4]. As to be expected, the disruption of the CNTF gene had no effect on the embryonic development of motoneurons and also, in the early postnatal phase, neither functional nor morphological changes of motoneurons were detectable [4]. However, starting with the 8th postnatal week, gradually increasing atrophic/degenerative changes accompanied by the formation of reactive microglial cells became apparent. At the age of 28 weeks, the number of motoneurons in the facial nucleus was reduced by 22% and, simultaneously, a very small but statistically significant reduction in the muscle strength as determined by "grip strength" measurement also became apparent. Future experiments have to demonstrate as to whether there is a further progress in the atrophic/degenerative changes or whether compensatory mechanisms, *e. g.* the production of brain-derived neurotrophic factor (BNDF), come into play as has been demonstrated after transection of the sciatic nerve [17].

Molecules expressed by skeletal muscle supporting motoneurons under experimental conditions

During the last year, a large number of molecules have been identified which are expressed in rat skeletal muscle during the critical period of embryonic development during which the physiological cell death and its target-mediated regulation takes place. These molecules are also, to a variable degree, expressed in the postnatal phase. They include members of the NGF gene family (BDNF, NT-3 and NT-4/5) (see [8, 18]), FGF-5 (a secretory member of the FGF gene family) [19], leukemia-inhibitory factor (LIF) [20] and insulin-like growth factor I (IGF- I) [8, 21]. Of the members of the NGF gene family, the most potent effects were exhibited by BDNF and NT-4/5, [8, 18] both acting via the trkB receptor. Neurotrophin-3 (NT-3) showed a small but nevertheless distinct effect both *in vitro* [8, 18] and *in vivo* (lesion experiments) [22]. In contrast, NGF did not support motoneurons either *in vitro* or *in vivo* under any experimental conditions [6, 8, 18]. The potency of the members of the NGF gene family in supporting the survival of rat motoneurons was parallelled by their efficiency in protecting motoneurons after nerve lesion [8, 22]. Moreover, it has been demonstrated that the administration of BDNF to the chick chorioallantoic membrane interferes with the physiological cell death of motoneurons in the chick embryo [23]. IGF-I which had previously been demonstrated to have a distinct sprouting effect on motoneurons *in vivo* (see [21]) is also expressed in skeletal muscle during embryonic development and in adulthood. After underestimation of its action in cultures of chick motoneurons [6] (due to the presence of horse-serum in the culture medium which contains inhibitory IGF-I binding proteins), IGF-I showed a marked effect although both with respect to potency and efficacy lower than BDNF in rat motoneurons cultured under serum-free conditions [8]. IGF-I also protected facial motoneurons after local administration of the lesioned facial nerve in newborn rats [8]. It remains to be established what the relative physiological functions of these molecules

are, and whether they reflect redundancy to secure the trophic support of an important system or whether a combination of various neurotrophic molecules is necessary to regulate the extent of their survival during embryonic development and the maintenance of their appropriate function in adulthood. The latter would represent an analogy to the recent observation that, for the survival of oligodendrocytes *in vitro*, a multiplicity of molecules is necessary, including NT-3, CNTF and IGF-I [24]. ▼

References

1. Hamburger V. 1958. Regression versus peripheral control of differentiation in motor hyperplasia. *Am. J. Anat.* 102: 365-410.

2. Hollyday M., Hamburger V. 1976. Reduction of the naturally occurring motor neuron loss by enlargement of the periphery. *J. Comp. Neurol.* 170: 311-20.

3. Sendtner M., Arakawa Y., Stöckli K. A., Kreutzberg G., Thoenen H. 1991. Effect of ciliary neurotrophic factor (CNTF) on motoneuron survival. *J. Cell Sci.* (suppl. 15): 103-9.

4. Masu Y., Wolf E., Holtmann B., Sendtner M., Brem G., Thoenen H. 1993. Disruption of the CNTF gene results in motoneuron degeneration. *Nature* 365: 27-32.

5. Thoenen H., Hughes R. A., Sendtner M. Trophic support of motoneuron physiological pathophysiological and therapeutic implications. *Exp. Neurol.* in press.

6. Arakawa Y., Sendtner M., Thoenen H. 1990. Survival effect of ciliary neurotrophic factor (CNTF) on chick embryonic motoneurons in cultures : comparison with other neurotrophic factors and cytokines. *J. Neurosci.* 10: 3507-15.

7. Bloch-Gallego E., Huchet M., El M'Hamdi H., Xie F. K., Tanaka H., Henderson C. E. 1991. Survival *in vitro* of motoneurons identified or purified by novel antibody-based methods is selectively enhanced by muscle-derived factors. *Development* 111: 221-32.

8. Hughes R. A., Sendtner M., Thoenen H. 1993. Representatives of several gene families influence survival of rat motoneurons *in vitro* and *in vivo*. Submitted.

9. Sendtner M., Kreutzberg G. W., Thoenen H. 1990. Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy. *Nature.* 345: 440-1.

10. Sendtner M., Stöckli K. A., Thoenen H. 1992. Synthesis and location of ciliary neurotrophic factor in the rat sciatic nerve of the adult rat after lesion and during regeneration. *J. Cell Biol.* 118: 139- 48.

11. Adler R., Land K. B., Manthorpe M., Varon S. 1979. Cholinergic neurotrophic factors : intraocular distribution of trophic activity for ciliary neurons. *Science* 204: 1434-6.

12. Nishi R., Berg D. K. 1979. Survival and development of ciliary ganglion neurones grown alone in cell culture. *Nature* 277: 232-4.

13. Lin L.-F. H., Mismer D., Lile J. D., Armes L. G., Butler III E. T., Vannice J. L., Collins F. 1989. Purification, cloning and expression of ciliary neurotrophic factor (CNTF). *Science* 246: 1023-5.

14. Mignatti P., Morimoto T., Rifkin D. B. 1992. Basic fibroblast growth factor, a protein devoid of secretory signal sequence, is released by cells via a pathway independent of the endoplasmic reticulum-Golgi complex. *J. Cell Physiol.* 151: 81-93.

15. Stöckli K. A., Lottspeich F., Sendtner M., Masiakowski P., Carroll P., Götz R., Lindholm D., Thoenen H. 1989. Molecular cloning, expression and regional distribution of rat ciliary neurotrophic factor. *Nature* 342: 920-3.

16. Schmalbruch H. 1984. Motoneuron death after sciatic nerve section in newborn rats. *J. Comp. Neurol.* 224: 252-8.

17. Meyer M., Matsuoka I., Wetmore C., Olson L., Thoenen H. 1992. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve : different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J. Cell Biol.* 119: 45-54.

18. Henderson C. E., Camu W., Mettling C., Gouin A., Poulsen K., Karihaloo M., Rullamas J., Evans T., McMahon S. B., Armanini M. P., Berkemeier L., Phillips H. S., Rosenthal A. 1993. Neurotrophins promote neuron survival and are present in embryonic limb bud. *Nature.* 363: 266-70.

19. Hughues R. A., Sendtner M., Goldfarb M., Lindholm D., Thoenen H. 1993. Evidence that fibroblast growth factor 5 is a major muscle derived survival factor for cultured spinal motoneurons. *Neuron* 10: 369-77.

20. Martinou J. C., Martinou I., Kato A. 1992. Cholinergic differentiation factor (CDF/LIF) promotes survival of isolated rat embryonic motoneurons *in vitro*. *Neuron.* 8: 737-44.

21. Caroni P., Becker M. 1992. The downregulation of growth-associated proteins in motoneurons at the onset of synapse elimination is controlled by muscle activity and IGF-I. *J. Neurosci.* 12: 3849-61.

22. Sendtner M., Schmalbruch H., Stöckli K. A., Carroll P., Kreutzberg G. W., Thoenen H. 1992. Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuronopathy. *Nature* 358: 502-3.

23. Oppenheim R. W., Qin-Wei Y., Prevette D., Yan Q. 1992. Brain-derived neurotrophic factor rescues developing avian motoneurons from cell death. *Nature* 360: 755-7.

24. Barres B. A., Schmid R., Sendtner M., Raff M. C. 1993. Multiple extracellular signals are required for long-term oligodentrocyte survival. *Development* 118: 283-95.