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The role of neurotrophins in the maintenance of the spinal cord motor neurons and the dorsal root ganglia proprioceptive sensory neurons

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Abstract

The aim of this study was to approach the question of neuronal dependence on neurotrophins during embryonic development in mice in a way other than gene targeting. We employed amyogenic mouse embryos and fetuses that develop without any skeletal myoblasts or skeletal muscle and consequently lose motor and proprioceptive neurons. We hypothesized that if, in spite of the complete inability to maintain motor and proprioceptive neurons, the remaining spinal and dorsal root ganglia tissues of amyogenic fetuses still contain any of the neurotrophins, that particular neurotrophin alone is not sufficient for the maintenance of motor and proprioceptive neurons. Moreover, if the remaining spinal and dorsal root ganglia tissues still contain any of the neurotrophins, that particular neurotrophin alone may be sufficient for the maintenance of the remaining neurons (i.e., mostly non-muscle- and a few muscle-innervating neurons). To test the role of the spinal cord and dorsal root ganglia tissues in the maintenance of its neurons, we performed immunohistochemistry employing double-mutant and control tissues and antibodies against neurotrophins and their receptors. Our data suggested that: (a) during the peak of motor neuron cell death, the spinal cord and dorsal root ganglia distribution of neurotrophins was not altered; (b) the distribution of BDNF, NT-4/5, TrkB and TrkC, and not NT-3, was necessary for the maintenance of the spinal cord motor neurons; (c) the distribution of BDNF, NT-4/5 and TrkC, and not NT-3 and Trk B, was necessary for the maintenance of the DRG proprioceptive neurons; (d) NT-3 was responsible for the maintenance of the remaining neurons and glia in the spinal cord and dorsal root ganglia (possibly via TrkB).

Keywords: MyoD; Myf5; Mouse embryo; BDNF; NT-3; NT-4/5; Receptors

1. Introduction

It is well established that during development motor neurons compete for their target, the skeletal muscle, to avoid programmed cell death. Human disorders characterized with motor control disturbances and human muscular diseases provide some evidence for that. However, it is still largely unclear whether any of the neurotrophins are of unique importance in preventing the death of developing motor neurons.

On the other hand, mounting evidence supports the notion that neurotrophins are indeed important regulators of the development and maintenance of the vertebrate nervous system. They are capable of promoting neuronal survival, proliferation and differentiation of precursor cells. They can induce morphological differentiation, enhance nerve regeneration, stimulate neurotransmitter expression or otherwise alter the physiological characteristics of neurons (reviewed by Apfel, 1999). These properties make neurotrophins promising as therapeutic agents.

There are two groups of neurotrophins influencing survival of sensory and motor neurons: (a) nerve growth factor (NGF) and (b) brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5). Other

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neurotrophic factors, such as ciliary neurotrophic factor (CNTF), glial cell derived neurotrophic factor (GDNF) and leukocyte inhibitory factor (LIF), also act on neuronal cells and appear to complement the effects of neurotrophins.

The survival of developing motor neurons depends on trophic factors derived from both, the target tissues (e.g., limb buds) and the central nervous system (Henderson et al., 1994; Oppenheim et al., 1995). The best candidates for physiological motor neuron trophic factors are the neurotrophins BDNF, NT-3, NT-4/5, because they are: (1) found in developing skeletal muscle during naturally occurring motor neuron cell death, (2) retrogradely transported by motor neurons and (3) observed to be important for spinal motor neuron survival in different experimental approaches (e.g., in avian embryo manipulation experiments) (Yan et al., 1992; DiStefano et al., 1992; Oppenheim, 1991; Oppenheim et al., 1992; Sendtner et al., 1992; Koliatsos et al., 1993, 1994; Henderson et al., 1993, 1994).

Neurotrophins exert their effects through binding to their receptors: p75, TrkA, TrkB and TrkC. Structurally, neurotrophins are dimeric proteins. Each of two subunits forming a neurotrophin has 120 aminoacid residues (Oppenheim, 1991). Basic residues are responsible for binding of particular neurotrophin to its high affinity tyrosine kinase receptor (Trk) located on the cell surface (Ibanez et al., 1993). On the other hand, a set of basic residues common to all neurotrophins is important for their binding to low affinity receptor designated p75 (Ryden et al., 1995).

Relationship between different skeletal muscle-associated neurons and target-derived neurotrophins BDNF, NT-3, NT-4/5 can be studied in different ways. In vitro studies suggest that survival of many populations of cranial sensory neurons firstly depends on BDNF or NT-3 (Vogel and Davies, 1991; Buchman and Davies, 1993). Apparently, E10 mouse trigeminal neurons, which have not innervated their target die within 48 h and can only be rescued by supplement of either BDNF or NT-3. During early stages of target field innervation, survival dependence switches to NGF (Buj-Bello et al., 1994). At E13 stage, dependence on BDNF switches to NGF, whereas, at E14 stage, dependence on NT3 switches to NGF.

The other possibility to study neurotrophic requirements of neurons is that neurotrophins, the receptor kinases and the p75 receptor component are targeted in null mutation experiments. In fact, up to 94–98% of motor neurons in the facial motor nucleus (FMN) were eliminated in *BDNF −/−:NT-4/5−/−*, *TrkB−/−* and *TrkB−/−:TrkC−/−* mice, whereas only 20–25% of motor neurons in the FMN were eliminated in *TrkC−/−* and *NT-3−/−* mice and 48–50% in *BDNF −/−* and *NT-4/5−/−* mice. In *BDNF −/−* and *NT3−/−* mice only 48–56% of spinal cord (SC) motor neurons were eliminated. Proprioceptive dorsal root ganglia (DRG) neurons were also eliminated depending on the genotype. *NT-4/5−/−*, *TrkC−/−* and *TrkB−/−* mice had the smallest loss of 15–20%, whereas *NT3−/−* mice lost up to 78% of DRG neurons. *BDNF −/−* mice lost 34% and *TrkB−/−:TrkC−/−* mice lost 51% of DRG neurons (Lee et al., 1992; Snider, 1994; Farinas and Reichardt, 1996; Snider and Silos-Santiago, 1996).

Although there is a little doubt that neurotrophins can prevent the death of motor neurons following injury, it is still unclear if BDNF, NT-3 or NT-4/5 is of unique importance in preventing the death (and therefore in the maintenance) of developing motor neurons (reviewed by Lewin and Barde, 1996). In the current report, we investigated the developmental relationship between skeletal muscle (i.e., control versus amyogenic embryos/fetuses), skeletal muscle-associated neuronal cell types (e.g., directly associated somatic motor neurons in the SC that innervate skeletal muscle and proprioceptive sensory neurons in the DRG that receive proprioceptive information from the muscle spindles) and the remaining SC and DRG tissues (i.e., amyogenic fetuses cannot maintain their muscle-innervating neurons, but they have the SC and DRG). Employing immunohistochemistry, we studied the distribution pattern of neurotrophins and their receptors in the SC and DRG of control embryos/fetuses (i.e., *Myf5+/−:MyoD+/−*) and compared it to the corresponding structures from double-mutant embryos/fetuses (i.e., *Myf5−/−:MyoD−/−*) lacking all skeletal muscles (Rudnicki et al., 1993) and consequently all somatic motor neurons in the SC and proprioceptive neurons in the DRG (Kablar and Rudnicki, 1999). We hypothesized that the normal distribution of a neurotrophin in the double-mutant SC/DRG could indicate its irrelevance to the maintenance of the motor and sensory neurons in the SC/DRG. Alternatively, the normal distribution of a neurotrophin in the double-mutant SC/DRG could indicate its relevance to the maintenance of the remaining motor and sensory neurons (mostly non-muscle-innervating) in the SC/DRG. In fact, we find the following: (a) during the peak of motor neuron cell death, the SC/DRG distribution of neurotrophins is not altered; (b) the distribution of BDNF, NT-4/5 and the receptors, and not NT-3, is necessary for the maintenance of the spinal cord motor neurons; (c) the distribution of BDNF, NT-4/5 and TrkC, and not NT-3 and Trk B, is necessary for the maintenance of the DRG proprioceptive neurons;(d) NT-3 is responsible for the maintenance of the remaining neurons and glia in the spinal cord and dorsal root ganglia.

2. Experimental procedures

2.1. Interbreeding and collection of embryos and fetuses

Embryos and fetuses lacking both *Myf5* and *MyoD* (designated as *Myf5−/−:MyoD−/−*, double-mutant or amyogenic) were derived by a two generation breeding scheme, as previously described (Rudnicki et al., 1993). In brief, *MyoD−/−* knock-out mice were bred with *Myf5+/−*