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Local and target-derived actions of neurotrophins during peripheral nervous system development

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Abstract. Neurotrophic factors are present in limiting quantities, and neurons that obtain an adequate supply of the required neurotrophic factor survive whereas those that compete unsuccessfully die. Analysis of null mutant mice for neurotrophins and Trk receptors as well as in vivo experiments in ovo in the chick applying exogenous neurotrophins or neutralising antisera have significantly increased knowledge of the roles they play during development. This review focuses on recent advances in understanding the various roles of neurotrophins in dorsal root ganglion sensory neuron development at different times in embryonic development – an early local role for differentiation of the sensory precursor cells and a later survival-promoting target-derived role for the mature neurons.

Key words. Neurotrophin; nervous system development; dorsal root ganglion; trophic; apoptosis.

Introduction

A number of early pioneering in vivo experiments studied the role of the prototypic neurotrophic factor, nerve growth factor (NGF), in the development of peripheral neurons. These experiments were based either on administration of excess NGF or on neutralising endogenous NGF using an anti-NGF antiserum. Exogenous administration of NGF to the chick embryo promotes survival of the dorsal root ganglion sensory neurons which would have otherwise died during the period of programmed cell death while neutralising NGF in vivo with an anti-NGF antibody leads to excessive cell death of dorsal root ganglion neurons. NGF acts on only a few of the sensory dorsal root ganglion neurons, yet programmed cell death during development and following target deprivation is widespread, suggesting a general involvement of neurotrophic factors regulating the survival peripheral neurons, and implying the presence of many more neurotrophic factors with similar functions.

The purification of brain-derived neurotrophic factor (BDNF) [1] ultimately led to the cloning of the gene and the discovery that NGF is a member of a gene family of structurally and functionally similar neurotrophic factors also including neurotrophin (NT)-3) and NT-4 in mammals. The neurotrophins contain a signal sequence for secretion, and are produced as proproteins a little over 200 amino acids long. Following proteolytic cleavage, a mature C-terminal active peptide slightly shorter than 120 amino acids long is released containing six cysteine residues at identically spaced positions in all mammalian neurotrophins. The mature part is very well conserved and approximately 50% of the amino acids are common to all neurotrophins. The neurotrophins are secreted as dimers associated by non-covalent bonds. Three different tyrosine kinase receptors mediate the effects of neurotrophins on neurons – TrkA, TrkB and TrkC. NGF binds and activates the TrkA receptor, BDNF and NT-4 share the signal-transducing receptor TrkB, and NT-3 binds and activates TrkC. Whereas NGF, BDNF and NT-4 show very little receptor promiscuity, NT-3 can under some circumstances also interact with the TrkA and TrkB receptors.

In recent years, mouse strains carrying null mutations in the genes encoding neurotrophins and their receptors have been generated. These mouse lines have provided an invaluable tool to analyse the roles of neurotrophins in the full physiological context of the living mouse, and with them, knowledge of the functions of all members in the neurotrophin family has increased rapidly. All the mutant mice except NT-4-null mice die shortly after birth and

have therefore been useful mostly for characterising the developmental roles of these neurotrophins in the peripheral nervous system. Analysis of the roles of neurotrophic factors in sensory neuron development using these mice strains and manipulations of chick embryos in ovo has led to the unexpected finding that NT-3 is required during gangliogenesis in virtually all populations of primary sensory neurons examined, including the dorsal root ganglion, trigeminal ganglion and the nodose/petrosal ganglion. The only known exception is the auditory ganglion, which acquires NT-3 dependency only after target innervation.

Early roles of NT-3: presence of both NT-3 and TrkC at the right time and location

The NT-3 homozygous mutant mice display close to a 60% loss of dorsal root ganglion neurons [2, 3]. Most of this loss occurs prior to target innervation and coincides with gangliogenesis between embryonic day (E)10.5 and E13 [4–6]. In fact, virtually all of this loss occurs during the critical period around E11, which coincides with the stage at which more than half of the dorsal root ganglion neurons are born in the mouse [7]. The early neuronal loss in NT-3–/– mice involves nonspecific partial loss in most or all subpopulations of sensory neurons [2] and in vivo experiments show that many but not all sensory neurons switch neurotrophin dependence later in development, possibly to NGF and BDNF dependence [8, 9]. Thus, this early loss precedes a classical target-derived period of neurotrophin action.

The early loss of neurons in NT-3–/– mice is consistent with a marked down-regulation of TrkC mRNA expression in the dorsal root ganglion just prior to the onset of the programmed cell death period which is controlled by a limiting quantity of target-derived neurotrophins and occurs in the mouse predominantly between E12 and E14. Most E11 dorsal root ganglion neurons express trkC, the NT-3 receptor. By E13, many of these cells have down-regulated TrkC [10] and have lost their responsiveness to NT-3 in culture [11, 12]. After birth, TrkC is only expressed in the largest dorsal root ganglion neurons comprising of approximately 20 % of the total population [13]. Consistently, survival dependency to NT-3 re-emerges at birth and the number of neurons sustained on NT-3 in vitro increases at least until 1 week after birth at which time close to 20% of the cultured neurons can be rescued by NT-3 [12]. Thus, the number of postnatal neurons that are supported by NT-3 in culture matches well the percentage of neurons expressing TrkC postnatally, and these most likely correspond to the slowly adapting sensory neurons innervating Merkel end-organs which are lost in the NT-3 mutant mice during the first week after birth (see target-derived roles below) [14].

E11 dorsal root ganglion neurons survive independently of NT-3 for at least 24 h in culture [15]. This 'independent' survival can be abrogated by neutralising endogenously produced NT-3 using an anti-NT-3 antiserum. Consistently, E11 dorsal root ganglion neurons from NT-3–/– mice do not survive in culture without trophic support [5]. RNAse protection assays have shown NT-3 mRNA expression in the E11 dorsal root ganglion neurons or associated mesenchymal tissue. Thus, NT-3 is expressed locally in the vicinity of the dependent neurons. X-gal staining of mice containing a LacZ gene introduced in the NT-3 locus has revealed, with cellular resolution, that NT-3 is expressed in the mesenchyme immediately adjacent to the ganglia and in proximal part of the limb at E11 [6]. At later stages expression was always detected along the tracts of the growing nerves. Taken together, both NT-3 and its receptor are present at the appropriate time and location during gangliogenesis.

Early functions of NT-3 in vivo and in vitro findings

A role for NT-3 in early development of sensory neurons has been established in cell culture experiments. Proliferation of cultured chick neural crest cells and early rat dorsal root ganglion cells can be stimulated by NT-3 [16, 17], and morphological maturation of isolated chick dorsal root ganglion neurons in culture is accelerated by the addition of NT-3 to the medium [18]. In the latter study, NT-3 was also observed to induce the generation of new neuronal profiles under serum-free conditions, indicating promotion of cell division. Administration of anti-NT-3 antibodies to the chick at the time of gangliogenesis leads to a dramatic loss of dorsal root and nodose ganglion neurons [19, 20], and the loss occurs in a population of precursor cells [20]. Thus, NT-3 is essential in vivo for a function other than regulating the survival of neurons. It could either stimulate the division of neuronal precursors or stimulate the differentiation of such precursors into NT-3-dependent neurons that die in the absence of NT-3. This early effect is specific to NT-3, since neutralising NGF at this stage does not affect neuronal numbers [19]. The reverse experiment – administration of exogenous NT-3 at the time of ganglion formation (between E3 and E6) in the chick – leads to a 35% and 50% reduction in the number of dorsal root and nodose ganglion neurons, respectively, which is accompanied by a reduction in the number of proliferating neuroblasts [21]. The reduction correlated with the period of neuroblast proliferation since injection of NT-3 between E6 and E9 in the chick led to a markedly increased number of neurons in the dorsal root ganglion, consistent with its survival-promoting effects on mature neurons [21]. These are significant findings, showing that peripheral nervous system cells respond to NT-3 during gangliogenesis in vivo and that

NT-3 may cause a cessation of precursor cell proliferation [21]. Thus, NT-3 could play dual functions during peripheral nervous system development, acting first in a local fashion as a proliferation stop and differentiation signal and later in development as a survival factor for selective functional populations of sensory neurons.

A number of studies have addressed the early role of NT-3 in mammals using null mutant mice. Excessive cell death in the dorsal root ganglion of mice that carry a deleted NT-3 gene was found to precede the period of programmed cell death, detected by the terminal dUTP nick end-labelling (TUNEL) method, and to cause a reduction in the number of proliferating precursor cells without altering the proportion of proliferating cells to total number of neurons, indicating that the absence of NT-3 leads to the death of proliferating sensory precursor cells [5, 8, 22]. Furthermore, the majority of proliferating cells detected by bromodeoxyuridine incorporation were also costained with the TUNEL method [5]. In contrast to neurons with an intact NT-3 gene, most cultured early embryonic NT-3–/– neurons as well as wild-type neurons when cultured with NT-3 neutralising antibodies die in the absence of exogenously added NT-3. This is consistent with the NT-3 expression data and suggests that cell death of early sensory neurons is prevented by a local action of NT-3 [5]. Similar results have been obtained in the studies of trigeminal gangion sensory neurons [6, 23]. Staining for a number of cell cycle proteins in NT-3 mutant mice shows that, similar to the chick data, sensory precursor cells fail to differentiate in these mice, override the G1 phase restriction point and die by apoptosis in S phase. In all instances, the dying cells double stain for G1 and early S phase markers [24]. The critical role of cell cycle re-entry in the cell death phenomenon was demonstrated by a cell cycle blocker that prevented early apoptosis in the NT-3 mutant mice [24].

Early roles of NT-3: influences on cell cycle control and cell death

Statin is a 57-kDa phosphoprotein abundantly expressed in only non-dividing quiescent cells and is localized to the nuclear envelope. Similarly, increased expression of $p27^{kip1}$ leads to inhibition of the activity of cyclin D and E-cdk complexes, cell cycle progression and induction of quiescence. The number of cells expressing these proteins is markedly elevated in the NT-3–/– mice reflecting an increase in the number of quiescent precursor cells [24]. An accumulation of statin-expressing cells would suggest that neuronal differentiation following their quiescence is compromised. Double staining for different markers reveals that most of these cells also express N-myc or the G1/S phase transcription factor E2F-1. Furthermore, p27-positive cells co-express proliferating cell nuclear antigen (PCNA), which is present only in S phase [24]. Interestingly, these dying cells can also be double-stained for neurofilament [6]. Together, these results suggest that differentiation is compromised and that these quiescent cells re-enter the cell cycle in the absence of NT-3. Thus, NT-3 appears to play similar roles in the chick and rodent: early cell cycle exit and differentiation. In the absence of NT-3, precursor cells become quiescent and express neuronal markers but fail to exit the cell cycle.

The cell death at early gangliogenesis is clearly caused by a mechanism different from the cell death seen in later neurotrophin-deprived neurons, because in the latter case, cell death is not accompanied by increases in expression of pro-cell cycle proteins [24]. C-myc over expression can overcome cell cycle arrest, block differentiation, and induce hyperproliferation and/or cell death [25–27]. During embryonic development, N-myc is expressed at high levels in the central nervous systems, in neural crest derivatives and in a few other cell types [28–30]. The nuclear expression of N-myc in dorsal root ganglion neurons is progressively restricted as the neurons differentiate and it accumulates in the cytoplasm instead. This loss of nuclear N-myc expression in the peripheral precursor cells is paralleled by, and could be a prerequisite for, their exit from the cell cycle and differentiation into neurons [31, 32]. The failure of cell cycle exit in NT-3–/– mice is preceded by a failure in nuclear N-myc down-regulation and is paralleled by activation of the full repertoire of G1 and S phase cell cycle proteins required for cell cycle re-entry [24]. The finding that the dying cells in the E11 NT-3–/– dorsal root ganglion display a persistent expression of nuclear N-myc suggests that NT-3 is either directly down-regulating nuclear N-myc expression and/or inhibiting a mitogenic pathway inducing nuclear N-myc expression. Interestingly, NGF has been shown to down-regulate N-myc expression and induce differentiation of neuroblastoma cell lines [33]. Thus, cell death of sensory precursor cells in the NT-3–/– mice may be intimately linked to the elevated levels of N-myc and G1/S phase cell cycle proteins and NT-3 could conceivably prevent cell death and induce cell cycle exit and differentiation of precursor cells by regulating N-myc in vivo, as NGF has been shown to down-regulate N-myc-expressing cultured neuroblastoma cells, and constitutively expressed N-myc prevents neuronal differentiation [33, 34].

Myc-induced proliferation and apoptosis are partly exerted by distinct downstream pathways and the concept of active suppression of myc-driven death via survival signals arose from studies showing that myc induces apoptosis in fibroblasts unless survival factors are present [25]. A mechanism of C-myc-induced apoptosis has recently emerged. The death pathway of C-myc requires a ligand-mediated activation of the cell death-inducing Fas or tu-

mour necrosis factor (TNF) receptors that generate intracellular apoptotic signals [27]. Myc sensitizes cells to death induced by Fas ligands through the transactivation of the Fas ligand and perhaps the Fas receptor [27, 35, 36]. Myc also promotes mitochondrial cytochrome c release [37]. It is intriguing that the common neurotrophin receptor, $p75^{NTR}$, like the Fas and TNF receptor contains an intracellular death domain [38–40] and mediates ligand-induced death in the developing nervous system [41, 42]. Thus, the excessive cell death in the early dorsal root ganglion of NT-3–/– mice is possibly caused by an N-myc sensitization to $p75^{NTR}$ receptor-induced cell death similar to that observed with Fas in fibroblast cells. The $p75^{NTR}$ promoter contains a proximal E-box that could bind N-myc. In a number of different neuronal cell lines, N-myc has indeed been found to transactivate the $p75^{NTR}$ receptor [unpublished results]. Therefore, the death of precursor cells that is associated with persistent N-myc expression is possibly mediated by transactivation of $p75^{NTR}$ leading to increased protein levels and signalling of the $p75^{NTR}$. In conclusion, these results point to a novel activity of neurotrophic factors in cell cycle control during early stages of development and suggest that an N-myc sensitisation to cell death in the nervous system is under the control of NT-3. Implications of these findings for normal development include the possibility that locally produced NT-3 can inflict constraints on differentiating precursor cells. Since the default of limiting NT-3 concentrations is an elimination of the precursors, NT-3 could affect the number of neurons generated during development of the peripheral nervous system. However, whether NT-3 is present in limiting quantities during early development is unclear.

Early roles of NT-3: receptor specificity

There is a large discrepancy in the neuronal numbers lost in NT-3–/– mice and trkC mutant mice. Whereas NT-3–/– mice show close to 60% loss of dorsal root ganglion neurons [2], TrkC mutant mice display only 17% loss [43, 44]. The loss in TrkC mutant mice includes all proprioceptive neurons [43, 44]. These and other findings have led to the suggestion that NT-3 supports many dorsal root ganglion neurons at early stages by acting through the NGF receptor trkA rather than through the preferred trkC receptor [45, 46]. In the study by Davies et al. [45], NT-3 was shown to promote the survival of embryonic sensory neurons derived from the TrkC null mutant mice. However, the concentration of NT-3 required was more than one order of magnitude higher than that sufficient to promote neurons containing trkC receptors. Thus, this in vitro experiment shows clearly that NT-3 can support survival of early sensory neurons via TrkA, albeit at high, possibly non-physiological concentrations. In another set of experiments White et al. [8] examined the timing of cell death in TrkA, TrkC and corresponding ligand null mutant mice and found a synchronous onset of NGF and TrkA survival dependence in the developing dorsal root ganglia. Increased cell death was seen at E13.5 in both NGF and TrkA null mutant mice whereas there was little cell death at E11.5. In contrast, both trkC and NT-3–/– mice showed a markedly increased cell death at E11.5. Thus, in these in vivo experiments, the onset of both NT-3 and TrkC dependence occurs simultaneously at E11.5. Similar results have been obtained in studies of the trigeminal sensory neurons [23, 47, 48]. NT-3 has also been proposed to elicit its early effects via trkB since a proportion of the dying cells in E11 NT-3–/– mice stain immunohistochemically for TrkB [9]. Although such an interaction might occur under physiological conditions [49], the similar number of dying cells at early stages in NT-3–/– mice and in mice deficient for its preferring receptor, TrkC [8], argues against a significant role of such an interaction during dorsal root ganglion development. Thus, these sets of experiments open up the possibility that NT-3 acts via TrkA and TrkB in the artificial situation where TrkC is absent. Under such conditions, endogenous levels of NT-3 are expected to increase due to absence of binding and internalisation of the ligand by TrkC.

Two studies with contradictory results suggest that NT-3 plays a different function during early development. Although TrkC mRNA in the rodent and TrkC mRNA and protein in birds are expressed in the majority of precursor cells during gangliogenesis as well as in migrating neural crest cells [10, 20, 50–52], the mRNA for Trk receptors is not translated in rodents, since Trk protein expression could not be detected in these cells using immunohistochemistry [9]. The loss of neurons in NT-3–/– mice was concluded to be caused by a non-specific premature differentiation of precursor cells [6]. Further studies are required to determine the significance of this discrepancy.

Target-derived roles of neurotrophins for sensory dorsal root ganglion neurons

The dorsal root ganglion processes many different kinds of information and the sensations it transduces are diverse. This is reflected by neurons differing in size, neuropeptide content, and functional properties, which have very diverse termination fields including skin, viscera and deep terminations within the muscle. There are three main distinct sensory modalities transduced by dorsal root ganglion neurons: nociception elicited by noxious or thermal stimulation, mechanoreception stimulated by mechanical stimulation of the skin, and proprioception elicited by mechanical displacement of the muscles and joints. Within each basal modality, there are many diffe-

rent submodalities. Each of these subpopulations of neurons requires a target-derived source of trophic support and have a remarkable specificity in requirements for the different members of the neurotrophin family. Such a specific function of neurotrophins for sensory neurons of different functional properties is consistent with the spatial expression pattern of the neurotrophins. NGF is expressed in skin, a major target of pain sensory neurons, and NT-3 is expressed in muscle spindles, Golgi tendon organs and Merkel cells, the targets of the NT-3-dependent neurons. A target-derived source of neurotrophins has also been demonstrated in the inner ear, in several other peripheral systems and in the brain. Therefore, the function of the neuron is often (but not always) matched by both the specific expression of a Trk receptor and by a localised expression of its ligand in the target (the sensory receptors) of that particular class of neurons.

Nociceptive neurons

About 45% of the adult lumbar dorsal root ganglion neurons express TrkA and these cells are mostly of small diameter [13, 53]. Using anti-NGF antibodies, NGF was shown to be required for survival of these nociceptive dorsal root ganglion neurons [54]. An NGF requirement for the survival of nociceptors is limited until postnatal day 2, whereafter depletion of NGF does reduce the cell number in the ganglion [55]. However, when anti-NGF was administered during the first 2 weeks after birth, high-threshold mechanoreceptors were largely lost without any loss of dorsal root ganglion neurons. This later effect is caused by a phenotypic switch of high to low-threshold mechanoreceptors (D hairs) which also conduct via A fibres [56]. Substance P is a neuropeptide produced in nociceptive neurons and is an important neurotransmitter in the transduction of pain. Embryonic anti-NGF treatment leads to a loss of substance P-containing dorsal root ganglion neurons, but not neurons with other cytochemical properties. Furthermore, the central fibres terminating in superficial lamina of the spinal cord where the pain-transducing fibres terminate are selectively lost [57]. These studies showing a specific loss of pain-transmitting sensory neurons following immunological deprivation of NGF by anti-NGF treatment [58, 59 and references therein] have been fully corroborated by genetic deletion of NGF in mice [60–62]. Thus, NGF clearly supports a functionally, cytochemically and anatomically discrete subpopulation of dorsal root ganglion neurons.

Although TrkA is expressed in approximately 50 % of the adult lumbar dorsal root ganglion neurons representing the small-calibre peptidergic neurons subserving nociception [13], more than 80% express the receptor during embryonic development [10, 57, 63, 64]. Consistently, close to 80% of the dorsal root ganglion neurons are lost in mice lacking NGF or TrkA [44, 60, 62, 65], including the substance P-expressing population as well as a non-peptidergic population of pain transmitting neurons. The adult non-TrkA-expressing neurons that have been established to express TrkA and depend on NGF only during embryogenesis are of the non-peptidergic class of nociceptors that bind the lectin IB4 [62]. Interestingly, these neurons were recently shown to switch from NGF dependency to a completely different class of trophic factors, glial cell line-derived neurotrophic factor (GDNF) postnatally [66, 67]. Thus, NGF acting through TrkA supports survival in the embryonic and neonatal animal of the small-calibre unmyelinated (C fiber) neurons belonging almost completely to the peptidergic subpopulation, whereas the population of non-peptidergic IB4 positive C fiber neurons depend on NGF only transiently during embryogenesis, and postnatally acquire a dependency on GDNF.

The loss of nociceptive neurons in NGF and TrkA null mutant mice results in marked consequences in behavioural tests for pain transduction. Consistent with a loss of nociceptive neurons in these mice, they display reduced pain sensitivity [60, 61]. Neutralising NGF in the adult animals also leads to altered pain sensitivity. In these experiments, detailed physiological properties were studied and NGF was found to influence sensitivity to thermal and chemical stimuli but not mechanical or noxious cold stimuli [68]. These changes could partly or fully be explained by a reduction of cutaneous innervation and altered receptive field following NGF neutralisation [68X–70].

Inflammation has both a neurogenic and as a non-neurogenic component [71]. Neurogenic inflammation is caused by the interplay between the pain-transmitting nerve terminal and immune and endothelial cells. Neurogenic inflammation does not occur in denervated human skin and can be prevented by a nerve block in rats [72, 73]. Neurogenic inflammation is dose-dependently related to peripheral levels of substance P/neurokinin A [74]. Substance P and calcitonin gene-related peptide released from the sensory nerve terminals induce inflammation by a number of mechanisms. Substance P stimulates vasodilation as well as contraction of endothelial cells inducing plasma extravasation [75]. Mast cells contain several inflammatory mediators known to excite primary afferents including histamine and serotonin. Substance P release by nerve terminals stimulates mast cell degranulation that contributes to sensitisation, and as could exert a positive reinforcement of substance P release from the nerve terminals. Systemic or local administration of exogenous NGF to animals produces hyperalgesia [76, 77]. Increased NGF levels are observed after inflammatory stimuli and result from increased expression and release of the factor. NGF is, thus, also involved in hyperalgesia associated with inflammation in the adult animals [78]. NGF also appears to participate in wind-up secondary to peri-

pheral changes in peptide expression that could increase neurotransmitter/modulator release from noxiceptor afferent terminals [79].

Mechanoreceptive neurons

BDNF promotes the survival of embryonic neurons at E11, the identity and phenotype of which is unknown. When examined in culture at E12–E18, there are no survival-promoting effects of BDNF. However, a survival promoting property re-emerges at P7 at which time approximately 35% of the dorsal root ganglion neurons can be rescued in culture [12, 80, 81]. These results are consistent with the studies of BDNF null mutant mice, where 35% of the dorsal root ganglion sensory neurons are lost postnatally [44, 82]. The BDNF receptor, TrkB, is expressed by a subpopulation of intermediate-size mechanoreceptive neurons [13, 83], indicating that the postnatal neuronal loss occurs in a subpopulation of mechanoreception-transmitting neurons. BDNF is also essential for normal mechanical sensitivity (but not survival) of low-threshold slowly adaptive mechanoreceptive terminals (Merkel end-organ terminals) that respond to skin indentation [83].

Although both BDNF and NT-4 bind TrkB present on responsive neurons, they activate different intracellular pathways and support distinct subpopulations of sensory neurons [83–86]. NT-4, but not BDNF, regulates the survival of a subclass of hair follicle receptors, the low-threshold D hair afferents that respond dynamically to skin stimulation [87].

NT-3 supports the postnatal survival of primary sensory neurons that mediate slowly adapting mechanoreception and their Merkel cell-containing touch dome end-organs. NT-3 homozygous null mutant mice show a gradual loss of Merkel end-organs and afferents starting shortly after birth, and at 2 week of age they are virtually absent. Heterozygous mutant mice also display a significant loss of Merkel end-organ afferents [14, 88]. This loss and the loss of neurons in the dorsal root ganglion can be rescued by over-expression of NT-3 in the skin under a kerating promoter [89]. Neutralising NT-3 using an antiserum in the postnatal mouse also leads to a loss of Merkel cells in the touch domes, whereas proprioceptive-transducing neurons are unaffected [90]. Thus, in contrast to the embryonic role of NT-3 in the development of the proprioceptive neurons (see below), NT-3 appears to be important for neuronal survival and maintenance of the slowly adaptive mechanoreceptive Merkel afferents only after they have established functional connections.

Proprioceptive neurons

The sensory end-organs of the proprioceptive dorsal root ganglion neurons are the intrafusal muscle fibres of the muscle spindles and the Golgi tendon organs. These specialized structures are induced by the ingrowing sensory axons [91]. Those that innervate the muscle spindle are termed type Ia and II, while type Ib innervates the Golgi tendon organ. Because the end-organs are induced by the ingrowing afferents, their absence results in a failure of induction. In NT3 knockout mice, muscle spindles never form. Furthermore, the Ia afferent central projection in the spinal cord that makes monosynaptic contacts with motor neurons is missing along with the proprioceptive dorsal root ganglion neurons identified by the cytochemical marker parvalbumin [2]. Mice lacking TrkC show, like NT-3–/– mice, a complete absence of Ia afferents and muscle spindles [43]. The loss of Ia neurons occurs very early in development, since the sensory fibers never reach the muscle in NT-3–/– mice [4]. NT-3 mutant mice also display a complete absence of γ-motor neurons that innervate the intrafusal muscle fibres. This loss is believe to be secondary to the loss of the proprioceptive dorsal root ganglion neurons and caused by an absence of its target, the intrafusal muscle fibres of the muscle spindles [4, 92]. NT-3 appears to be the only neurotrophin on which these neurons depend, since neither NGF, BDNF nor NT-4 null mutant mice show the pronounced phenotype resulting from a loss of muscle spindle innervation as seen in the NT-3–/– mice, i.e., loss of co-ordination of movement of the limbs and limbs that frequently are locked in an extensor posture.

NT-3 is expressed in the immediate adjacent tissues of the dorsal root ganglion, later in development along the growing nerves, and after muscle spindle innervation, in the intrafusal muscle fibres of the muscle spindles [5, 6, 93]. NT-3 is also expressed throughout development in the central target of the proprioceptive neurons, the spinal cord motor neurons, [6, 10]. Thus, since proprioceptive neurons establish direct contact with these motor neurons they could be a possible source of NT-3 along with the peripherally expressed NT-3. However, proprioceptive neurons of NT-3–/– mice can be rescued by over expressing NT-3 in the muscle under a myogenin promoter [94], showing that peripheral NT-3 is sufficient to promote their survival. Similar results have been obtained in the chick embryo: administration of NT-3 rescues the proprioceptive neurons that are lost by limb bud extirpation, seen as the presence of Ia projection terminating in the ventral horn of the spinal cord. Consistently, anti-NT-3 treatment peripherally but not centrally leads to reduced Ia projection [95]. Limb bud extirpation results in a loss of virtually all motor neurons. Delivery of NT-3 peripherally is sufficient to prevent the loss of the proprioceptive neurons and the Ia projection even in the absence of motor neurons in chick embryos with limb bud amputation [96]. Thus, NT-3 produced peripherally is essential for proprioceptive neurons whereas NT-3 produced by spinal cord motor neurons may not be necessary for normal development of these neurons.

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