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Non Target-Derived Roles of the Neurotrophins

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Non target-derived roles of the neurotrophins

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SUMMARY

The hypothesis that target-derived neurotrophic factors are essential for the survival, differentiation and maintenance of sensory, sympathetic and motor neurons has been well supported by analysis of mice bearing null mutations in the neurotrophins and their receptors. However, the localization of brain-derived neurotrophic factor (BDNF) in a population of dorsal root ganglia (DRG) sensory neurons (Ernfors *et al.* 1990*b*; Ernfors & Persson 1991; Schecterson & Bothwell 1992) suggested the additional possibility that BDNF could act in a paracrine or autocrine manner to mediate neuronal survival. We tested this hypothesis in cultured adult DRG neurons, which survive as single cells in microwells in the absence of added trophic factors (Lindsay 1988). About 35 % of these neurons were specifically killed by BDNF antisense oligonucleotide administration in a dose-dependent manner, with no effect of sense oligonucleotides. Antisense administration was accompanied by an 80 % decrease in BDNF protein levels over the first 24 h of treatment (Acheson *et al.* 1995). The BDNF autocrine loop that we propose to be present in sensory neurons may be representative of a broader phenomenon in the nervous system as a whole, where the balance of neurotrophic support may shift during development from target-derived to paracrine or autocrine modes. Perhaps as a consequence of this developmental shift, the survival of both peripheral nervous system (PNS) and central nervous system (CNS) neurons in the adult is less affected by axotomy or target removal when compared to their response during development.

1. NGF AS A MODEL OF A TARGET-DERIVED TROPHIC FACTOR

Nerve growth factor (NGF) has served as the prototypical target-derived trophic factor, and what has been learned about NGF expression and function has formed the basis for many of the general assumptions that are made about the role of neurotrophic factors in development (Levi-Montalcini 1987; Barde 1994; Lindsay *et al.* 1994). Indeed, NGF is present in the target tissues of responsive peripheral nervous system (PNS) neurons (Heumann *et al.* 1984), and elimination of NGF through the use of targeted null mutations (Crowley *et al.* 1994) or neutralizing antibodies during development (Johnson *et al.* 1980; Carroll *et al.* 1992; Ruit *et al.* 1992) leads to the loss of NGF-responsive sympathetic and neural crest-derived sensory neurons. Similarly, increased expression of NGF in skin, driven by the keratin promoter, at a stage when NGF levels normally decrease, results in the rescue of NGF-responsive sympathetic and trigeminal sensory neurons from naturally occurring cell death (Albers *et al.* 1994). Thus both classical studies and more recent knockout and transgenic studies lead to the conclusion that target-derived NGF determines the survival of a specific population of responsive neurons. Target ablation leads to neuronal death through elimination of the trophic support provided by NGF; conversely, increased target size or increased expression of NGF within the target leads to increased neuronal survival, even above normal levels. NGF can thus clearly act as a regulator of naturally occurring cell death in the peripheral nervous system.

2. BDNF: AN NGF-RELATED NEUROTROPHIN

Brain-derived neurotrophic factor (BDNF) was first identified as a survival factor for several types of PNS sensory neuronal populations which did not respond to NGF (Barde *et al.* 1982; Lindsay *et al.* 1985). BDNF's subsequent purification and cloning led to the elucidation of a gene family which now consists of four members, NGF, BDNF, NT-3 and NT-4/5 (Leibrock *et al.* 1989; Ernfors *et al.* 1990*a*; Hohn *et al.* 1990; Jones & Reichardt 1990; Maisonpierre *et al.* 1990; Rosenthal *et al.* 1990; Berkemeier *et al.* 1991; Hallböök *et al.* 1991; Ip *et al.* 1992). Almost at the same time, it was shown that the high-affinity receptors for the neurotrophins were a family of tyrosine kinase receptors, the Trks (Martin-Zanca *et al.* 1989; Kaplan *et al.* 1991; Klein *et al.* 1991; Lamballe *et al.* 1991; Soppet *et al.* 1991; Squinto *et al.* 1991; Klein *et al.* 1992). BDNF and NT-4/5 both bind with high affinity to TrkB, whereas TrkA functions mainly as a receptor for NGF and TrkC as a receptor for NT-3 (for review see Glass & Yancopoulos 1993).

Initially, it was assumed that BDNF would conform to the NGF model, and be shown to function as a target-derived factor for any identified responsive neuronal populations. BDNF was shown to act *in vitro* on a wide spectrum of both placode- and neural crest-derived sensory neurons, as well as on motor neurons (Lindsay *et al.* 1985; Davies *et al.* 1986; Hohn *et al.* 1990; Wong *et al.* 1993). However, data regarding the expression of BDNF and TrkB, together with data from the recently generated mice null in BDNF has led to re-

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evaluation of the role of BDNF as a target-derived factor, and has forced a broadening of our ideas about how BDNF functions in development.

3. SOMETIMES BDNF DOES APPEAR TO FUNCTION AS A TARGET-DERIVED FACTOR, BUT WITH AT LEAST ONE CAVEAT

Mice null in the BDNF gene (BDNF −/−) are lacking vestibular neurons (Ernfors *et al.* 1994*a*; Jones *et al.* 1994; Conover *et al.* 1995; Liu *et al.* 1995). A careful developmental analysis indicates that the full complement of neurons is present at early stages of development, but once vestibular neurons reach their target hair cells, they begin to die (L. Bianchi, R. M. Lindsay & G. D. Yancopoulos, unpublished observations). These findings are consistent with the idea that target-derived BDNF regulates the survival of vestibular neurons.

The caveat to this straightforward interpretation of the data is that, at least in chicken, vestibulocochlear neurons express TrkB extremely early, from the time when they can be initially recognized as a ganglion (Dechant *et al.* 1993*a*). Similarly, neuroepithelial cells of the developing otocyst express BDNF from very early stages in development (Dechant *et al.* 1993*a*). Thus it would seem that vestibulocochlear neurons should be able to respond to BDNF from the very beginning of their developmental time course, provided that they have access to it. The fact that developing neurons can ignore the fact that BDNF has been knocked out until mid-stages of development implies that: (i) BDNF protein is not made or is not secreted; (ii) secreted BDNF is somehow sequestered until later in development; (iii) TrkB protein is not made or is not functional; or (iv) signalling elements needed for BDNF signal transduction cascades are not present until the target has been reached. At any rate, there appears to be more complicated modulation of the BDNF response system beyond the level of receptor or factor expression.

4. EVIDENCE THAT BDNF IS NOT SIMPLY A TARGET-DERIVED FACTOR

Various trophic factors have been localized within many populations of central nervous system (CNS) neurons, consistent with their possible role as target-derived factors for afferent inputs. However, dorsal root ganglion sensory neurons do not have afferent inputs in the periphery, making the role of trophic factors expressed in these neurons less clear. It is possible that sensory neurons provide neurotrophins to the neurons which they project to centrally. A subpopulation of DRG neurons express BDNF, as determined by in situ hybridization, although neither the precise proportion of neurons expressing BDNF nor the time-course of BDNF expression during development have been established (Ernfors *et al.* 1990*b*; Ernfors & Persson 1991; Schecterson & Bothwell 1992). Neuronal expression within the dorsal root ganglia (DRG) could be indicative of an autocrine or paracrine role for BDNF. Such as role gains further theoretical support from the fact that when sensory neurons are axotomized in the adult, the degree of cell death is minimal compared to the profound loss seen after axotomy early in development. It may be that early in development, sensory neurons depend to such an extent on their peripheral and/or central targets for trophic support to mediate their survival that target deprivation alone (axotomy) leads to cell death. Later in development, autocrine mechanisms may become the predominant source of neurotrophic support, such that axotomy is no longer fatal to these neurons. This may be a widespread phenomenon among mature neurons, because the survival of both PNS and CNS neurons in the adult is much less affected by axotomy or target removal when compared to their response during development.

5. BDNF AS AN AUTOCRINE FACTOR

We have recently demonstrated that BDNF acts as an autocrine survival factor for a population of cultured adult rat DRG neurons (Acheson *et al.* 1995). Adult rat DRG neurons have the interesting property of surviving in culture without added trophic factors (Lindsay 1988). Indeed, adult DRG neurons can survive in microwells as single neurons (Lindsay 1988). One explanation for this apparent lack of trophic factor dependence would be that the cells are making their own trophic factors.

Autocrine loops have been successfully interrupted in transformed cells by application of antisense oligonucleotides (Zamecnik & Stephenson 1978; Agris *et al.* 1986; Zamecnik *et al.* 1986). Short oligonucleotides are readily taken up into cells by an unknown mechanism (Wu-Pong *et al.* 1992) and have been shown to interfere with the production of the relevant autocrine factor for varying periods of time, ranging from hours to days (Becker *et al.* 1989; Morrison 1991). The result of antisense oligonucleotide application in these models is to inhibit proliferation, which can be restored either by adding the same growth factor exogenously or by adding an alternative mitogen (Becker *et al.* 1989; Morrison 1991). We used BDNF antisense oligonucleotides (18-mers) from the 3′ end of the coding sequence corresponding to the carboxyl terminal region of the mature protein (3′-AS) to interfere with BDNF translation. Treatment of cultured DRG neurons with these oligonucleotides resulted in an 80% decrease in BDNF protein levels over the first 24 h in culture, as well as a 35% decrease in the number of surviving neurons over a 72 h time course. Exogenously added BDNF rescued virtually all of the antisense-susceptible DRG neurons (Acheson *et al.* 1995).

Although these data suggested that BDNF produced locally in adult DRG cultures may be involved in an autocrine loop which mediates neuronal survival, they did not rule out possible paracrine support between neurons. Alternatively, antisense oligonucleotides may have produced the neuronal loss we observed by

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depletion of BDNF from the non-neuronal cells in the cultures, which are known to produce BDNF. Potentially to rule out either of these possibilities, we cultured enriched DRG neurons as single cells. When single neurons were treated with BDNF antisense oligonucleotides, the same proportion of neurons (35%) died in response to treatment as had been seen in the mixed neuron-non-neuronal cell cultures. These data suggested that the presence of non-neuronal cells was not required for the effect of antisense to become apparent. Single neuron experiments also showed that the majority of neurons susceptible to BDNF antisense oligonucleotide-mediated death could be rescued by exogenous BDNF (Acheson *et al.* 1995).

We have also examined the effect of BDNF antisense oligonucleotides in BDNF null mice. We first established the time course of growth factor-autonomous survival of DRG neurons, which begins about 10 days after birth, and is fully expressed by P15. When P15 DRG neurons were examined from BDNF null mice, there was no effect of BDNF antisense oligonucleotides in culture. As BDNF null mice have lost up to 30% of their DRG neurons, we reasoned that the BDNF-dependent neurons had already died in these mice (Acheson *et al.* 1995).

Our finding that about 35% of adult DRG neurons depend on an autocrine supply of BDNF for survival correlates strongly with recent data indicating that 30–35% of adult DRG neurons express transcripts encoding full-length TrkB (McMahon *et al.* 1995). Interestingly, NT-3 was also able to rescue most of the antisense-susceptible neurons. Rescue of BDNF antisense oligonucleotide-sensitive neurons by NT-3 suggests either that NT-3 activates TrkB in this system (Glass & Yancopoulos 1993) or that TrkB and the preferred NT-3 receptor, TrkC, are coexpressed on the affected neuronal population. The latter interpretation of our results is supported by recent data obtained using *in situ* hybridization which indicates that the majority of TrkB-expressing neurons in adult DRG also express TrkC (McMahon *et al.* 1995).

The BDNF autocrine loop that we propose to be present in sensory neurons may be representative of a broader phenomenon among both PNS and CNS neurons, where the balance of neurotrophic support shifts during development from target-derived to paracrine or autocrine. For example, motor neurons, which respond to NT-3 during development also express NT-3 mRNA (Ernfors & Persson 1991; Schecterson & Bothwell 1992; Wong *et al.* 1993), and substantia nigra neurons which respond to BDNF and NT-3 express high levels of BDNF and NT-3 mRNA (Hyman *et al.* 1991; Gall *et al.* 1992). A further example is hippocampal neurons, which in the adult express high levels of mRNA for all of the neurotrophins (Kokaia *et al.* 1993) and during development respond to BDNF and NT-3 (Ip *et al.* 1993).

What triggers the autocrine loop? BDNF is already expressed at P1. However, the time course of autocrine survival is such that it first begins to be seen in rat at P11. It could be that TrkB expression regulates the onset of the autocrine loop. Very few (6%) DRG neurons are TrkB positive at E18 (Mu *et al.* 1993), but in the adult, 30% of cells are TrkB positive (McMahon *et al.* 1995). It remains to be determined precisely when in development DRG neurons die in either TrkB or BDNF null mutant mice.

Are there other autocrine loops? Only about 30% of adult DRG neurons in culture appear to utilize BDNF to keep themselves alive, whereas at least 85% of the neurons survive without added factors. It would thus appear that other autocrine or paracrine loops exist, at least in cultured neurons, unless some of the neurons have become truly 'trophic factor independent'.

6. NT-3: BOTH TARGET-DERIVED AND NON-TARGET DERIVED ROLES?

At first sight, data from NT-3 null mice would appear to support the concept of NT-3 acting as a target-derived factor. In these mice, both proprioceptive neurons and their target, the spindle apparatus in muscle, are missing at birth (Ernfors *et al.* 1994*b*; Fariñas *et al.* 1994). However, a more careful developmental analysis suggests that some of the proprioceptive neurons are either never born or die very early in development, before they reach their targets. Similarly, some sympathetic neurons are eliminated in NT-3 null mice (Fariñas *et al.* 1994), and can be eliminated in newborn rats with a function-blocking anti-NT-3 antiserum (Zhou & Rush 1995). These results are consistent with an action of NT-3 on very early precursor cells in the sympathetic ganglia (DiCicco-Bloom *et al.* 1993), as has also been suggested in the chicken system (Dechant *et al* 1993*b*). Studies with developing auditory neurons also support the notion that NT-3 is important early in development, before targets have been reached, because auditory neurons die very early in development in NT-3 null mice. Thus, NT-3 may act early in development on a broad range of neurons and/or neuronal precursors, and may not function at all as a target-derived factor later in development.

7. CONCLUSIONS

The neurotrophin family consists of NGF, its prototypical member, as well as BDNF, NT-3 and NT-4/5 (Barde 1994; Lindsay *et al.* 1994). High-affinity receptors, the Trks, have been described for all of the neurotrophin family members, and p75, first described as the low affinity NGF receptor, binds all of the neurotrophins as well as NGF (Barker & Murphy 1992; Chao 1992). The responses of various neuronal populations to the different neurotrophins depends on the complement of Trks and p75 that are expressed. It is now becoming clear that sensory neurons change their Trk expression throughout development, thus respond differentially to neurotrophins at distinct developmental stages.

In very early developmental stages, sensory neurons undergo a trophic-factor independent stage which may involve autocrine production of neurotrophins (Vogel & Davies 1991; Wright *et al.* 1992; Davies & Wright 1995). As the neurons begin to grow towards their targets, the pattern of Trks expressed may change. The

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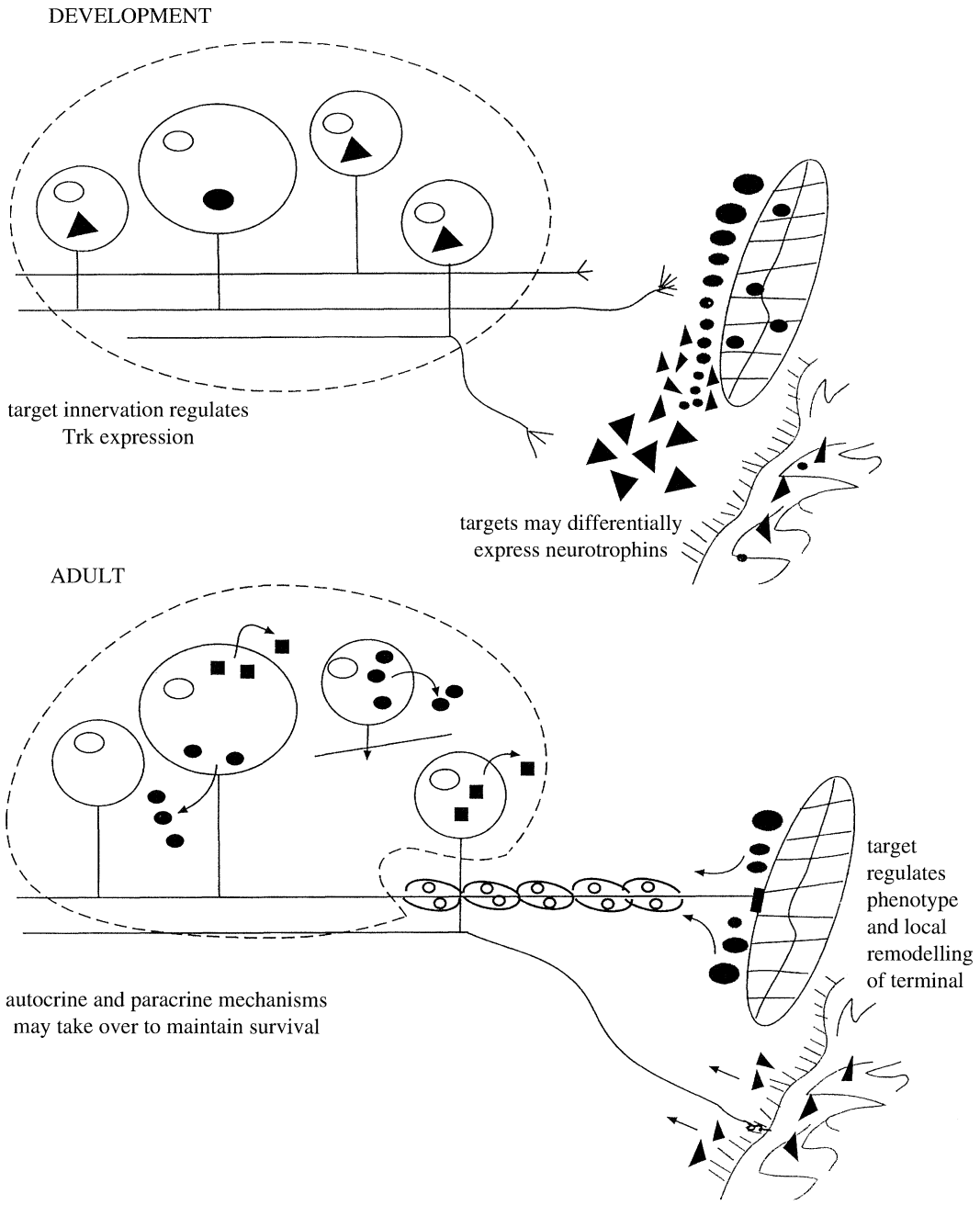

Figure 1. Changing roles of neurotrophins throughout sensory neuron development. In early development, neurons encounter neurotrophins along their growth pathways. Trk expression may be different during the growth phase versus the target-interaction phase of sensory neuron development. In adulthood, neurons express both Trks and neurotrophins. Target-derived factors may influence neuronal phenotype and sprouting, whereas survival may be mediated by autocrine or paracrine mechanisms within the ganglia.

growing neurites encounter neurotrophins along their pathways, and this may provide them with information that tells them where they are and/or whether to keep growing. When the target is reached, Trk expression may be regulated further, and target-derived neurotrophins may communicate different messages to the neurons, including survival as well as differentiation and maturation (figure 1).

If the target tissue produces more than one neurotrophin, as in the example of skin, which produces both NGF and NT-3, these two molecules could act as partial antagonists to one another. Competition for limiting amounts of neurotrophin in the target, together with potential spatial cues provided by different point sources of NT-3 and NGF acting antagonistically, ultimately determine which neurons survive the period of naturally occurring cell death and the nature of their terminal arborizations.

In the adult, multiple Trks are expressed on many (if not most) sensory neurons, thus making them capable of responding to neurotrophins produced in different locations. For example, a Trk A-/TrkB-positive neuron may respond both to NGF from its target and to BDNF supplied in a paracrine or autocrine fashion by other

neurons in the ganglion (figure 1). Autocrine neurotrophins may regulate survival, as our *in vitro* data suggests, whereas target-derived NGF may regulate peptide levels or other phenotypic markers.

The traditional role of target-derived factor which fits NGF so well does not fully describe the complicated roles of BDNF and NT-3 in sensory neuron development. Although these factors are indeed produced in target tissues, they are also produced in sensory neurons themselves, and are thus likely to act as autocrine or paracrine factors influencing both survival and other functional properties of the neurons. An understanding of the expanded role of these factors in development necessitates a careful analysis of the developmental sequences of Trk expression as well as neurotrophin expression.

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