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Review

Studies of neurotrophin biology in the developing trigeminal system

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ABSTRACT

The accessibility of the primary sensory neurons of the trigeminal system at stages throughout their development in avian and mammalian embryos and the ease with which these neurons can be studied in vivo has facilitated investigation of several fundamental aspects of neurotrophin biology. Studies of the timing and sequence of action of neurotrophins and the expression of neurotrophins and their receptors in this well characterised neuronal system have led to a detailed understanding of the functions of neurotrophins in neuronal development. The concepts of neurotrophin independent survival, neurotrophin switching and neurotrophin cooperativity have largely arisen from work on the trigeminal system. Moreover, in vitro studies of trigeminal neurons provided some of the first evidence that the neurotrophin requirements of sensory neurons are related to sensory modality. The developing trigeminal system has been studied most extensively in mice and chickens, each of which has particular advantages for understanding different aspects of neurotrophin biology. In this review, I will outline these advantages and describe some of the main findings that have arisen from this work.

Key words: Apoptosis; sensory neurons; neurotrophin receptors.

NEUROTROPHINS AND NEUROTROPHIC THEORY

Nerve growth factor (NGF) is the founder member of a family of secreted proteins that includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), neurotrophin-4/5 (NT4/5) and neurotrophin-6 (NT-6). Work on NGF laid the foundations of the neurotrophic theory, the idea that the survival of developing neurons is dependent on a supply of a neurotrophic factor from the tissues they innervate. Classic studies of the effects of manipulating the availability of NGF to neurons in the developing nervous system (by administering exogenous NGF or function-blocking anti-NGF antibodies) together with studies of the sites of NGF synthesis showed that the survival of sympathetic neurons and certain kinds of sensory neurons is dependent on a supply of NGF from the tissues they innervate. A wealth of in vitro and in vivo studies of the effects of NGF and other neurotrophins on neurons and studies of mice with targeted null mutations in genes coding for these proteins and their receptors have extended the generality of the neurotrophic hypothesis. In addition to their well established role in promoting and regulating the survival of developing neurons, neurotrophins also affect neuron precursor cell proliferation and differentiation, regulate neurotransmitter and neuropeptide synthesis and influence neuronal form and synaptic function throughout life (Averbuch et al. 1994; Davies, 1994; Thoenen, 1995; Lewin & Barde, 1996).

Two kinds of transmembrane glycoproteins are receptors for neurotrophins: members of the Trk family of receptor tyrosine kinases which are receptors for different neurotrophins and p75 which is a common receptor for all neurotrophins. Trk receptor tyrosine kinases undergo rapid transphosphorylation following ligand binding, leading to a cascade of

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protein phosphorylations in the cell. Expression studies in cell lines have shown that TrkA is a receptor for NGF, TrkB is a receptor for BDNF and NT4/5 and TrkC is a receptor for NT3, although NT3 can also bind and signal less efficiently via TrkA and TrkB (Barbacid, 1994; Kaplan & Stephens, 1994; Bothwell, 1995). Several observations suggest that Trks mediate the survival-promoting actions of neurotrophins in developing neurons. First, the distinctive neuronal deficiencies in mice with null mutations in the *trkA*, *trkB* and *trkC* genes are similar to those observed in mice with null mutations in the *NGF*, *BDNF* and *NT3* genes, respectively (reviewed by Davies, 1994; Snider, 1994; Lewin & Barde, 1996). Second, ectopic expression of TrkA receptors in TrkB-expressing neurons confers an NGF survival response on these neurons (Allsopp et al. 1993), whereas ectopic expression of TrkB in TrkA-expressing neurons confers a BDNF survival response (Ninkina et al. 1996).

The functions of p75 are more diverse and complex than those of the Trks. In vitro studies of neurons obtained from p75 null mice have shown that p75 is not required for the survival response of neurons to neurotrophins. However, the dose responses of p75-deficient embryonic sensory and postnatal sympathetic neurons to NGF are shifted to higher NGF concentrations, suggesting that p75 specifically enhances the sensitivity of NGF-dependent neurons to NGF (Davies et al. 1993*b*; Lee et al. 1994*b*). Interestingly, the sensitivity of neurons to other neurotrophins is not reduced in p75-deficient neurons. These findings accord with the phenotype of p75 null mice which is consistent with a partial loss of NGF-dependent sensory and sympathetic neurons (Lee et al. 1992, 1994*a*).

p75 also plays a role in ligand discrimination by the Trk receptors. The ability of NT3 to activate TrkA in the PC12 pheochromocytoma cell line is enhanced when NT3 binding to p75 is prevented by function-perturbing antibodies or when p75 expression is very low (Benedetti et al. 1993; Clary & Reichardt, 1994). Likewise, sympathetic neurons from postnatal p75-deficient mice are more sensitive to NT3 than sympathetic neurons from wild type animals (Lee et al. 1994*b*). These findings suggest that p75 reduces the ability of NT3 to signal via TrkA. Furthermore, studies with mutated BDNF and NT4/5 proteins that bind TrkB normally but fail to bind p75 suggest that p75 plays a role in TrkB ligand discrimination. Whereas the BDNF mutant activates TrkB as effectively as wild type BDNF, the NT4/5 mutant activates TrkB less effectively than wild type NT4/5 (Ryden et al. 1995).

In addition to the evidence that p75 selectively modulates the trophic actions of neurotrophins in neurons expressing different Trk receptors, several experimental findings suggest that p75 is able to promote cell death in the absence of ligand. In a neuron-like cell line and in PC12 cells grown in serum-free conditions without NGF, p75 expression increases the rate of cell death (Rabizadeh et al. 1993). Antisense p75 oligonucleotides that reduce p75 expression promote the survival of postnatal rat dorsal root ganglion (DRG) neurons grown in the absence of NGF, suggesting that at this stage of development p75 is able to act as a constitutive death signal in the absence of NGF (Barrett & Bartlett, 1994). In addition, recent evidence suggests that in certain cells lacking TrkA, NGF exerts a cytotoxic effect by binding to p75 (Casaccia-Bonnefil et al. 1996; Frade et al. 1996).

THE TRIGEMINAL SYSTEM AND ITS ADVANTAGES

The ease with which specific populations of neurons and their progenitor cells can be obtained for experimental studies from the peripheral nervous system is the main reason why much of our understanding of the cell biology of neuronal development and the role of neurotrophins has come from studying this division of the nervous system. One of the best characterised developing sensory systems is the trigeminal. The 2 populations of primary sensory neurons of this system are those of the trigeminal ganglion and the trigeminal mesencephalic nucleus (TMN). The trigeminal ganglion innervates mainly mechanoreceptors, thermoreceptors and nociceptors in the face, oral cavity and nasal cavity. Its central axons terminate on several groups of second order sensory neurons in the brainstem. The TMN innervates muscle spindles and tendon organs in the muscles of mastication. Its central axons terminate on trigeminal motoneurons and several other groups of neurons in the brainstem.

Developing trigeminal neurons have been studied most extensively in rodent and chicken embryos, each of which has particular experimental advantages. In both classes of vertebrates, the trigeminal ganglion is the largest sensory ganglion in the embryo and provides a ready source of neurons for in vitro studies from the earliest stages of sensory neuron development. This has facilitated studies of the actions of neurotrophins and the regulation of receptor expression at successive stages of neuronal development. The maxillary and mandibular processes of the 1st

branchial arch, which form a major component of peripheral innervation territory of the trigeminal ganglion, are clearly defined structures that can be easily dissected out prior to and during the earliest stages of their innervation. This has not only been invaluable for studying the influence of these tissues on the early growth and guidance of trigeminal axons, providing the first experimental evidence for chemotropism in the developing nervous system (Lumsden & Davies, 1983, 1986), but has permitted quantitative studies of neurotrophin expression in target tissues.

A major advantage of the mouse trigeminal system has been afforded by the advent of transgenic mice. The generation of null mutations in genes encoding the neurotrophins and their receptors and the consequences of the expression of neurotrophin transgenes have provided powerful new approaches to test and extend the conclusions of earlier in vitro studies of trigeminal neurons.

The rodent trigeminal system is one of the best characterised and extensively studied model systems for investigating the establishment of topographic neural projections. The characteristic pattern of whisker follicles on the snout is replicated in the brainstem, thalamus and somatosensory cortex by corresponding arrays of multineuronal units. The use of mouse transgenic techniques for manipulating neurotrophin expression are also beginning to make important contributions to our understanding of the potential roles of these proteins in the formation of topographic maps (Henderson et al. 1993).

Essential for the design and interpretation of studies to understand the functions of neurotrophins at different developmental stages is an accurate chronology of the phases of neuronal development in the experimental system under investigation. This has been obtained for the trigeminal ganglion and its innervation of the periphery from detailed light and electron microscopic studies of closely staged mouse embryos (Davies & Lumsden, 1984, 1986; Davies, 1987*a*; Pinon et al. 1996). These studies have shown that the earliest axons emerge from the trigeminal ganglion at E9.5. The peripheral axons grow to their targets at about 20 μm per hour and come into proximity with the epithelium by E10.5 in the mandibular process and E11 in the maxillary process. The number of neurons in the ganglion peaks at E13 and decreases by 50% to reach a stable number by birth as a result of neuronal apoptosis which peaks at E14.

In contrast to the mammalian system, most TMN neurons in birds are concentrated within the tectal commissure which can be easily and cleanly dissected from the adjoining tissues. Because of their large size, TMN neurons can be separated from all other cells by differential sedimentation, resulting in an essentially pure preparation of proprioceptive neurons (Davies, 1986). This has permitted comparative studies of the neurotrophin requirements of these neurons and the predominantly cutaneous sensory neurons of the trigeminal ganglion. In birds, the trigeminal ganglion contains 2 distinctive, anatomically segregated populations of neurons: the large diameter, placode-derived neurons are located in the ventrolateral (VL) part of the ganglion and the small-diameter, neural crest-derived neurons are located in the dorsomedial (DM) part of the ganglion.

EARLY SURVIVAL REQUIREMENTS OF MOUSE TRIGEMINAL NEURONS

Some of the most extensive information on the survival requirements of neurons at different stages in their development has come from detailed in vitro and in vivo studies of embryonic mouse trigeminal ganglion neurons. The observation that neurites emerge from trigeminal ganglion explants grown in neurotrophin-free medium at the stage when the earliest axons emerge from these ganglia in vivo led to the proposal that neurons survive and extend axons independently of neurotrophins during the earliest stages of their development (Davies et al. 1981; Davies & Lumsden, 1984). This proposal was strengthened by studies of other populations of sensory neurons (Ernsberger & Rohrer, 1988; Davies, 1989; Vogel & Davies, 1991) and sympathetic neurons (Coughlin & Collins, 1985; Ernsberger et al. 1989) grown in dissociated culture at early developmental stages. Moreover, the demonstration that many early DRG neurons survive in single cell cultures for 24 h in chemically defined medium without neurotrophins (Wright et al. 1992) indicates that these neurons do not obtain factors from other cells in order to survive. Although the duration of neurotrophin independent survival of the earliest trigeminal neurons is probably long enough for their axons to reach their peripheral targets (Buchman & Davies, 1993), it is likely that the survival of later generated trigeminal neurons is dependent on trophic support from the tissues through which their axons grow to reach their peripheral targets. As discussed below, this support is probably mediated by BDNF and NT3 which are synthesised by these intervening tissues.

After the period of neurotrophin independence, in vitro studies have shown that most trigeminal neurons are first dependent on BDNF or NT3 for survival and

subsequently become dependent on NGF for survival. When trigeminal neurons are grown at very low density in defined medium at E10, the stage when the earliest trigeminal axons are starting to grow to their peripheral targets, the majority of these neurons die after 24 h unless either BDNF or NT3 are present in the culture medium. BDNF and NT3 exert a direct survival promoting action on differentiated neurons in these cultures because almost all of the neurons in cohorts identified shortly after plating are still alive after 48 h in cultures containing these neurotrophins (Buchman & Davies, 1993; Paul & Davies, 1995). In contrast to BDNF and NT3, NGF has a negligible effect on the survival of E10 trigeminal neurons in culture. However, in cultures established at successively later stages, NGF supports an increasing proportion of the neurons. Concomitant with the acquisition of NGF survival dependence, responsiveness to BDNF and NT3 is rapidly lost in all but a small subset of neurons. During the switchover period there is negligible additional neuronal survival in cultures containing NGF plus BDNF or NT3, indicating that the neurons pass through a transitional phase when they are capable of responding to each of these neurotrophins (Buchman & Davies, 1993). The acquisition and loss of responsiveness to different neurotrophins is not a simple on–off phenomenon occurring at a particular stage in development. As neurons mature in vivo, the length of time they are able to survive with BDNF or NT3 in vitro decreases with age, whereas the length of time they are able to survive with NGF increases with age (Paul & Davies, 1995). The dose responses of the neurons to BDNF and NT3 also shift by several orders of magnitude to higher concentrations with age (Buj-Bello et al. 1994).

Studies of the timing of neuronal death in the trigeminal ganglia of embryos that have null mutations in genes encoding the neurotrophin and their receptors have confirmed the physiological relevance of in vitro studies of neurotrophin switching. In *trkB*$^{-/-}$ embryos, the number of trigeminal neurons undergoing apoptosis is markedly increased during the early developmental stages when neurons are responsive to BDNF in vitro. In *trkA*$^{-/-}$ embryos, neuronal apoptosis is markedly elevated later in development when neurons are responsive to NGF in vitro (Pinon et al. 1996). Thus many neurons depend on TrkB signalling for survival in the early trigeminal ganglion before becoming dependent on TrkA signalling. Although early trigeminal neurons survive in culture equally well with either of the 2 preferred TrkB ligands, BDNF and NT4 (Davies et al. 1993*a*), BDNF appears to be the relevant TrkB ligand in vivo because there is a significant reduction in the number of neurons in the trigeminal ganglia of *BDNF*$^{-/-}$ neonates (Ernfors et al. 1994*a*; Jones et al. 1994) but not *NT4*$^{-/-}$ neonates (Conover et al. 1995; Liu et al. 1995).

The number of neurons in the trigeminal ganglia of *NT3*$^{-/-}$ embryos is also markedly reduced before the peak of naturally occurring neuronal death (ElShamy & Ernfors, 1996; Wilkinson et al. 1996), implying that like BDNF, endogenous NT3 is also required at an early stage in trigeminal ganglion development. However, there are conflicting views on the role of NT3 in the ganglion during this stage. It has been reported that the majority of the dying cells in the early trigeminal ganglia of *NT3*$^{-/-}$ embryos had incorporated BrdU administered 5 h earlier and expressed nestin, a marker for precursor cells. This together with finding that there is a reduction in the number of proliferating cells in early trigeminal ganglia of *NT3*$^{-/-}$ embryos led to the conclusion that NT3 promotes the survival of proliferating precursor cells (ElShamy & Ernfors, 1996). In contrast, the number of neurons and nonneuronal cells (mostly precursor cells) is unchanged in the trigeminal ganglia of *NT3*$^{-/-}$ embryos compared with wild type embryos during the earliest stages of gangliogenesis at E10.5, and the subsequent rapid depletion of neurons initially occurs without significant change in the number of nonneuronal cells and proliferating cells in the ganglion, suggesting that NT3 acts as a survival factor for at least a proportion of early neurons (Wilkinson et al. 1996). A reduction in the trigeminal precursor cell population of *NT3*$^{-/-}$ embryos was, however, observed towards the end of the period of neurogenesis (Wilkinson et al. 1996).

TrkC, the preferred NT3 receptor, is unlikely to mediate the effects of NT3 in the early trigeminal ganglion because there is not a marked loss of neurons in the early trigeminal ganglia of *trkC*$^{-/-}$ embryos (Pinon et al. 1996). Because the survival of early trigeminal neurons depends on TrkB and not TrkA signalling (Pinon et al. 1996) and because NT3 is able to promote the survival of embryonic sensory neurons by signalling via TrkB (Davies et al. 1995), it is likely that NT3 acts predominantly via TrkB early on.

Although there is clear evidence for a switch or change in neurotrophin survival requirements of mouse trigeminal and DRG neurons (Farinas et al. 1997) and populations of neural crest-derived neurons in the chicken embryo (Buj-Bello et al. 1994), several populations of placode-derived sensory neurons do not appear to switch responsiveness between neurotrophins during the early stages of their development. For example, the BDNF-dependent neurons of the

ventrolateral part of the embryonic chicken trigeminal ganglion do not show early survival responses to NGF or NT3, and the BDNF-responsive and NT3-responsive nodose neurons do not show an early response to NGF (Buj-Bello et al. 1994).

In trying to understand the purpose of neurotrophin switching, it may be significant that populations of sensory neurons that switch dependence from BDNF/NT3 to NGF survive only briefly without neurotrophins in culture during the earliest stages of their development (Ernsberger & Rohrer, 1988; Wright et al. 1992; Buchman & Davies, 1993) whereas sensory neurons that do not switch have a regulated period of neurotrophin independence that is correlated with target distance (Vogel & Davies, 1991). Because the peripheral axons of sensory neurons that undergo a switch in neurotrophin dependence appear to be exposed to BDNF and NT3 en route to their peripheral targets (Arumae et al. 1993; Buchman & Davies, 1993; White et al. 1996; Farinas et al. 1997), it is likely that these neurotrophins play a role in sustaining neurons before their axons reach the peripheral tissues where NGF is produced. Sensory neurons that do not exhibit a switch in neurotrophin dependence survive in vitro for different lengths of time without neurotrophins during the early stages of their development; neurons with more distant targets survive longer than neurons with nearby targets (Vogel & Davies, 1991). It is possible, therefore, that these populations of neurons are not dependent on intermediate support before encountering their targets.

NEUROTROPHIN RECEPTOR EXPRESSION IN MOUSE TRIGEMINAL NEURONS

The acquisition and loss of responsiveness to different neurotrophins is associated with changes in the expression of the corresponding receptors. TrkB mRNA is expressed at very low levels in the trigeminal ganglion during the period of neurotrophin independence when the sensory earliest axons are growing towards their targets (Ninkina et al. 1996). The onset of BDNF dependence is associated with increased expression of trkB transcripts encoding the full-length receptor, whereas loss of BDNF responsiveness is associated with decreased expression of these transcripts and the increasing expression of trkB transcripts coding for variants that lack the catalytic tyrosine kinase domain and function as negative modulators of BDNF signalling in neurons (Ninkina et al. 1996).

TrkA and p75 are expressed at very low levels in the trigeminal ganglion before the axons reach their targets (Wyatt & Davies, 1993; Schropel et al. 1995). The acquisition of a sustained NGF survival response is associated with a marked increased expression of these receptors in trigeminal neurons (Wyatt & Davies, 1993). Similar temporal patterns of trk transcripts have also been described in the developing chicken embryo trigeminal ganglion (Williams et al. 1995).

Although numerous studies have shown that NGF increases TrkA and p75 expression in neurons and cell lines (Doherty et al. 1988; Cavicchioli et al. 1989; Higgins et al. 1989; Lindsay et al. 1990; Fusco et al. 1991; Miller et al. 1991; Kojima et al. 1992; Verge et al. 1992; Wyatt & Davies, 1993, 1995; Miller et al. 1994; Verdi & Anderson, 1994), the finding that the increase in trkA and p75 mRNA expression that accompanies the onset of a sustained NGF survival response in developing trigeminal neurons is unaffected in $NGF^{-/-}$ embryos (Davies et al. 1995) suggests that target-derived NGF is not involved in regulating NGF receptor expression and dependence during this period of development. In vitro studies suggest that other molecules are required for the acquisition of NGF dependence and the loss of BDNF and NT3 dependence. When trigeminal neurons are cultured before they respond to NGF, they survive with BDNF well beyond the switchover period from BDNF to NGF dependence, and when these early neurons are switched from BDNF to NGF after various times in culture they die as rapidly as neurotrophin-deprived neurons. However, neurons switched from BDNF or NT3 to NGF in cultures set up at stages throughout the switchover period exhibit an NGF survival response that improves with age (Paul & Davies, 1995). Thus, unlike the onset of BDNF dependence which is controlled by an intrinsic timing mechanism in early sensory neurons (Vogel & Davies, 1991, 1993), these results suggest that the switch from BDNF/NT3 to NGF dependence is due to signals that act on the neurons during the switchover period.

NEUROTROPHIN EXPRESSION IN THE MOUSE TRIGEMINAL SYSTEM

Accompanying the changing survival requirements of trigeminal neurons are matching changes in the expression of neurotrophins. BDNF and NT3 mRNAs are expressed in the peripheral trigeminal territory prior to the arrival of the earliest sensory axons (Arumae et al. 1993; Buchman & Davies, 1993) and NGF mRNA and protein are expressed later with the arrival of sensory axons (Davies et al. 1987). The

levels of BDNF and NT3 mRNAs are initially highest in the mesenchyme through which the axons grow to the periphery (Arumae et al. 1993; Buchman & Davies, 1993; Wilkinson et al. 1996), whereas NGF mRNA is expressed predominantly in the target field epithelium and to a lesser extent in the subjacent mesenchyme (Davies et al. 1987). Later in development, NT3 mRNA is largely confined to the oral epithelium and the mesenchyme underlying the skin (Wilkinson et al. 1996). Interestingly, the level of BDNF mRNA expression is very low and NT3 mRNA is undetectable in the developing hindbrain, suggesting that the periphery is the major source of neurotrophins for developing trigeminal ganglion neurons (Arumae et al. 1993; Buchman & Davies, 1993; Wilkinson et al. 1996).

Studies of regional differences in the levels of NGF mRNA in the cutaneous epithelia of the trigeminal territory during the early stages of its innervation has provided evidence in support of a key assumption of the neurotrophic hypothesis (Harper & Davies, 1990). At the onset of neuronal death in the trigeminal ganglion there are marked differences in the concentration of NGF mRNA in the epithelia of the maxillary, mandibular and ophthalmic territories that are correlated with final innervation density. This suggests that the level of NGF production in target field cells governs the number of neurons that survive. However, because the same percentage cell death occurs in each of the subsets of trigeminal neurons that innervate the maxillary, mandibular and ophthalmic territories (Davies & Lumsden, 1984), regional differences in NGF synthesis are not responsible for establishing differences in innervation density, rather they maintain differences that arise earlier in development.

NEUROTROPHIN SPECIFICITY IN THE CHICKEN TRIGEMINAL SYSTEM

The first studies of the neurotrophin requirements of proprioceptive neurons were facilitated by the development of methods to isolate, purify and culture TMN neurons from chicken embryos (Davies, 1986). The survival of these neurons is unaffected by NGF (Davies et al. 1987) and is promoted by BDNF (Davies et al. 1986*b*) and a muscle-derived factor (Davies, 1986) that turned out to be NT3 (Hohn et al. 1990). The demonstration that 2 different factors support the survival of TMN neurons and that these factors seem to potentiate at low concentrations provided some of the first evidence to suggest that the survival of certain populations of neurons may be regulated by multiple neurotrophic factors (Davies et al. 1986*a*).

The conclusions of in vitro studies of TMN neurons have been confirmed by studying the phenotype of mice with null mutations in genes encoding neurotrophins and their receptors. Neuron counts in the TMN of neonatal mutant mice have shown that, compared with wild type mice, there is an approximate 50% reduction in $BDNF^{-/-}$ mice (Ernfors et al. 1994*a*; Jones et al. 1994) and $NT3^{-/-}$ mice (Ernfors et al. 1994*b*). These results suggest that both BDNF and NT3 contribute to the survival of TMN neurons in vivo. Fibre tracing studies have shown that $trkC^{-/-}$ mice do not possess group Ia muscles afferents in the spinal cord (Klein et al. 1994) and examination of the soleus muscle in $NT3^{-/-}$ mice has revealed the complete absence of muscle spindles (Ernfors et al. 1994*b*). The lack of effect of NGF on proprioceptive neuron survival, originally observed in cultured TWN neurons (Davies et al. 1987), has been confirmed in studies of $trkA^{-/-}$ and $NGF^{-/-}$ phenotypes. In both mutants, small diameter neurons were lost, leaving large diameter DRG neurons unaffected (Crowley et al. 1994; Smeyne et al. 1994).

During the second half of embryonic development, the DM and VL neurons of the chicken trigeminal ganglion exhibit distinctive survival responses to neurotrophins. Whereas NGF promotes the survival of the majority of DM and has negligible effect on VL neurons (Ebendal & Hedlund, 1975; Davies & Lumsden, 1983), BDNF promotes the survival of the majority of VL neurons and has a negligible effect on DM neurons (Davies et al. 1986*b*) and NT3 has a negligible effect on either population (Hohn et al. 1990; Pinon et al. 1995). In vitro studies of trigeminal neurons and other populations of cranial sensory neurons in the chicken embryo led to the conclusion that the neurotrophin requirements of developing sensory neurons are related to sensory modality (Davies, 1987*b*). This conclusion has since been confirmed and extended by elegant and detailed studies of the Trk receptors expressed by different kinds of DRG neurons (Mu et al. 1993; McMahon et al. 1994) and by studies of the kinds of sensory neurons eliminated in mice with null mutations in genes encoding neurotrophins (Crowley et al. 1994; Enfors et al. 1994*a*, *b*; Jones et al. 1994; Conover et al. 1995; Liu et al. 1995) and their receptors (Klein et al. 1993, 1994; Smeyne et al. 1994).

Studies of chicken embryo trigeminal neurons have also been useful in determining which kinds of neurons express transcripts for neurotrophins themselves. In addition to the expression of neurotrophins in sensory

neuron target fields, BDNF mRNA and NT3 mRNA have been localised by in situ hybridisation to a proportion of the neurons in embryonic dorsal root ganglia (Ernfors et al. 1990; Ernfors & Persson, 1991; Schecterson & Bothwell, 1992; Zhang et al. 1994). These findings have led to the suggestion that BDNF and NT3 could be acting by an autocrine mechanism on developing sensory neurons. Direct evidence for an autocrine action of BDNF in sensory neurons has come from using antisense BDNF oligonucleotides to interfere with the production of BDNF in cultured sensory neurons. This approach was first used in DRG neurons cultured before their axons have reached their targets (Wright et al. 1992). At this stage, the neurons survive in isolation in defined medium without added neurotrophins and undergo a maturational change from having a small, spindle-shaped, phase-dark cell body and short neurites to developing a larger, phase-bright, spherical cell body and long neurites. This maturational change is accelerated by BDNF and is largely inhibited by antisense BDNF oligonucleotides (Wright et al. 1992), suggesting that BDNF acts by an autocrine route to promote the maturation. These antisense oligonucleotides do not affect the survival of early DRG neurons, suggesting that BDNF does not act by an autocrine mechanism to promote neuronal survival at this stage (Wright et al. 1992). This experimental approach has also been used to demonstrate that BDNF acts by an autocrine mechanism to promote the survival of a subpopulation of adult DRG neurons (Acheson et al. 1995). Adult DRG neurons survive independently of neurotrophins when cultured in isolation (Lindsay, 1988) and antisense BDNF oligonucleotides reduce the number of surviving neurons by 35% (Acheson et al. 1995).

The demonstration that BDNF acts on sensory neurons by an autocrine mechanism during the early and late stages of their life history when these neurons survive independently of added neurotrophins (Wright et al. 1992; Acheson et al. 1995) raised the question as to whether a BDNF autocrine loop also operates during the phase of naturally occurring neuronal death. Measurements of the levels of BDNF mRNA in highly purified preparations of embryonic chicken trigeminal neurons showed that the NGF-dependent DM neurons express the highest levels of BDNF mRNA, the VL neurons which contain few NGF-dependent neurons express lower levels of BDNF mRNA and the TMN neurons which contain no NGF-dependent neurons do not express detectable levels of BDNF mRNA (Robinson et al. 1995). This, together with the demonstration that DM neurons synthesise and release biologically active BDNF (Robinson et al. 1996), suggests that during the period of naturally occurring neuronal death, BDNF is expressed predominantly by NGF-dependent neurons and hence cannot act by an autocrine route in the majority of sensory neurons.

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