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Expression and function of TrkB variants in developing sensory neurons

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Mouse trigeminal neurons survive independently of neurotrophins when their axons are growing to their targets, and are then transiently supported by BDNF before becoming NGF dependent. During the stage of neurotrophin independence, transcripts encoding the BDNF receptor, TrkB, were expressed at very low levels. During the stage of BDNF dependence, high levels of a transcript encoding a receptor with the catalytic tyrosine kinase domain were expressed. Although the levels of this transcript fell as the neurons lost responsiveness to BDNF, there were concomitant increases in the expression of transcripts encoding TrkB variants lacking the kinase domain. Analysis of RNA from purified neurons showed that all of these transcripts were present in neurons. BDNF and NGF up-regulated the expression of these transcripts early in development but had little effect later on. To test whether truncated TrkB modulates BDNF signalling via catalytic TrkB, we injected TrkB expression plasmids into NGF-dependent sympathetic neurons. Whereas expression of catalytic TrkB alone conferred a BDNF survival response, co-expression of non-catalytic TrkB substantially reduced this response. Our results suggest that BDNF responsiveness in sensory neurons during development is modulated by the relative levels of catalytic and non-catalytic TrkB.

Keywords: brain-derived neurotrophic factor/nerve growth factor/trigeminal neurons/TrkB

Introduction

In the developing vertebrate peripheral nervous system, many neurons depend for their survival on a supply of one or more neurotrophins from the tissues they innervate (Davies, 1994). These secreted proteins exert their effects on neurons by binding to members of the Trk family of receptor tyrosine kinases (Barbacid, 1994; Klein, 1994). Expression studies in cell lines have shown that TrkA is the receptor for nerve growth factor (NGF), TrkB is the receptor for brain-derived neurotrophic factor (BDNF) and neurotrophin 4/5 (NT4/5), and TrkC is the receptor for NT3 (Barbacid, 1994; Klein, 1994). Binding of a neurotrophin to its receptor leads to phosphorylation of the kinase domain which initiates a cascade of protein phosphorylations in the cell (Kaplan and Stephens, 1994). Alternative splicing generates TrkB and TrkC variants that lack a tyrosine kinase domain (Klein *et al.*, 1990a,b; Lamballe *et al.*, 1993; Tsoulfas *et al.*, 1993). However, the function of these non-catalytic variants is unclear.

At different stages of development, neurons acquire and lose dependence on particular neurotrophins (Davies, 1994). To understand how these changes in neurotrophin dependence are regulated, it is necessary to determine the time course of neurotrophin receptor expression and elucidate the signals that control the expression of these receptors. One of the best characterized populations of neurons with respect to its developmental requirements for neurotrophins are the sensory neurons of the embryonic mouse trigeminal ganglion. These neurons survive independently of neurotrophins when their axons are growing to their targets, and can be supported in culture by BDNF, NT3 or NT4/5 at the stage when their peripheral axons come into proximity with developing cutaneous tissues. Shortly afterwards, the neurons lose responsiveness to these neurotrophins and become dependent on NGF for survival (Davies and Lumsden, 1984; Buchman and Davies, 1993; Davies *et al.*, 1993; Paul and Davies, 1995). These developmental changes in neurotrophin requirements are mirrored by differences in the timing of neurotrophin expression in the trigeminal territory (Davies *et al.*, 1987; Arumae *et al.*, 1993; Buchman and Davies, 1993). The demonstration that neuronal death in the trigeminal ganglia of *trkB*$^{-/-}$ embryos peaks much earlier than in wild-type and *trkA*$^{-/-}$ embryos (Pinon *et al.*, 1996), together with the finding that *BDNF*$^{-/-}$ mice (Ernfors *et al.*, 1994; Jones *et al.*, 1994), but not *NT4*$^{-/-}$ mice (Conover *et al.*, 1995; Liu *et al.*, 1995), have marked reductions of the numbers of neurons in the trigeminal ganglion, suggest that the early *in vitro* survival response of trigeminal neurons to BDNF is physiologically relevant.

Work on other populations of cranial sensory neurons suggests that the onset of BDNF dependence is controlled by an intrinsic timing mechanism in neurons that becomes programmed in their progenitor cells (Vogel and Davies, 1991, 1993). In contrast, the switch from BDNF dependence to NGF dependence appears to be effected by signals that act on the neurons during the switch-over period *in vivo* (Paul and Davies, 1995). The acquisition of the NGF survival response is correlated with a marked increase in the expression of *trkA* mRNA in the neurons (Wyatt and Davies, 1993) and the labelling of the neurons by iodinated NGF (Davies *et al.*, 1987). NGF does not, however, affect the expression of *trkA* mRNA in cultured trigeminal neurons (Wyatt and Davies, 1993) and is not required for the increase in *trkA* expression *in vivo* that occurs shortly after these neurons start innervating their targets (Davies *et al.*, 1995).

The main purpose of the present study was to understand how BDNF responsiveness is switched on and off in

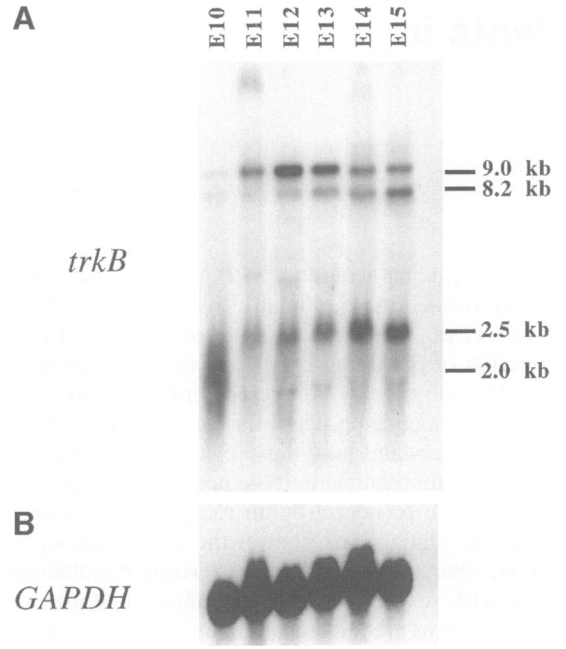

Fig. 1. Autoradiograms of a Northern blot of total RNA extracted from E10–E15 trigeminal ganglia hybridized first with a ^{32}P-labelled *trkB* nick-translated probe (**A**) and second with a nick-translated *GAPDH* probe (**B**). The locations of the 2.0, 2.5, 8.2 and 9.0 kb *trkB* bands are shown in (A). Very similar hybridization signals for *GAPDH* are observed in each lane in (B), indicating that approximately the same amount of total RNA was run in each lane.

development by studying the timing of expression of *trkB* transcripts in embryonic trigeminal neurons and by examining the function of different TrkB variants expressed in neurons. In addition, we wished to ascertain whether neurotrophins regulate the expression of *trkB* transcripts at different developmental stages.

Results

Time course of trkB mRNA expression in trigeminal ganglia

To determine which *trkB* transcripts are expressed in trigeminal ganglia and how the relative levels of expression of these transcripts change in development, we hybridized Northern blots of total RNA extracted from E10–E15 trigeminal ganglia with a *trkB*-specific ^{32}P-labelled DNA probe. We used a probe derived from the extracellular domain of *trkB* cDNA as this should hybridize to all known *trkB* mRNA variants (Klein *et al.*, 1990a,b).

Three *trkB* transcripts were expressed prominently in developing trigeminal ganglia: 9.0, 8.2 and 2.5 kb (Figure 1). From previous work, it is known that the 9.0 kb transcript codes for a TrkB receptor that possesses a catalytic tyrosine kinase domain, and the 8.2 and 2.5 kb transcripts code for truncated receptors that lack the tyrosine kinase domain (Klein *et al.*, 1990a). In addition, a minor 5.5 kb transcript that codes for a TrkB receptor that possesses a tyrosine kinase domain (Klein *et al.*, 1990a) was also detected. The previously described 8.0 and 2.0 kb transcripts, which code for truncated receptors (Klein *et al.*, 1990a), were not detected in developing trigeminal ganglia, except at E10 where the 2.0 kb transcript was expressed. The 8.0 and 2.0 kb transcripts

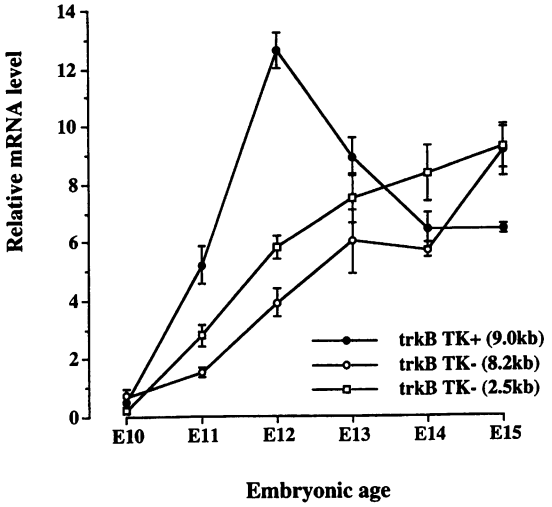

Fig. 2. Graph showing the relative levels of the 9.0 (catalytic) and 8.2 and 2.5 kb (non-catalytic) *trkB* transcripts obtained by densitometric measurements of Northern blots of E10–E15 trigeminal ganglion RNA. The mean and SEM of three separate measurements are shown.

were not detectable in E14 whole head RNA (not shown) but were expressed in adult brain (not shown). The 2.0 and 2.5 kb transcripts were also detected in RNA from non-neural tissues such as spleen RNA.

To compare the relative levels of *trkB* transcripts expressed at different ages, similar amounts of total RNA from trigeminal ganglia of different ages were run in adjoining lanes. After hybridizing Northern blots with the *trkB* probe, the blots were re-probed with a nick-translated probe for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*; which codes for a housekeeping protein) to standardize total RNA levels for densitometric comparison of the relative levels of *trkB* transcripts expressed at different ages. Figure 2 shows the combined results of densitometric comparison of the relative levels of 9.0, 8.2 and 2.5 kb transcripts from three separate sets of Northern blots of E10–E15 trigeminal ganglion RNA. At E10, the levels of all three transcripts were very low. The level of the 9.0 kb transcript encoding the catalytic receptor increased markedly between E10 and E12. After peaking at E12, the level of this transcript gradually decreased from E12 to E15. The levels of the 8.2 and 2.5 kb transcripts gradually increased from E10 to E15. Thus, at E11 and E12, the level of the 9.0 kb transcript was higher than that of the 8.2 and 2.5 kb transcripts, whereas at E13 the levels of the three transcripts were similar, and by E15 the 8.2 and 2.5 kb transcripts predominated.

Neurons express mRNA encoding full-length and truncated TrkB

To determine which of the three main *trkB* transcripts was detected in RNA from whole ganglia, differential sedimentation (Davies, 1986) was used to separate neurons from the other cells in the ganglion. This was carried out at E15 because the size difference between neurons and non-neuronal cells is sufficiently great at this stage to enable preparations of >95% pure neurons to be obtained. Figure 3 shows an autoradiogram of a Northern blot of total RNA extracted from purified neurons and total ganglion cells hybridized with the extracellular domain *trkB* probe. The neurons clearly expressed 9.0, 8.2 and

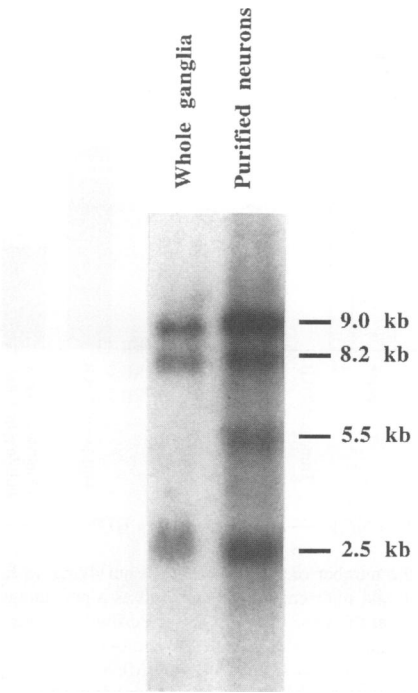

Fig. 3. Autoradiogram of a Northern blot of total RNA extracted from purified E15 trigeminal ganglion neurons (>95% neurons) and total trigeminal ganglion cells (neurons plus non-neuronal cells) hybridized with the ^{32}P-labelled *trkB* nick-translated probe. The locations of the 2.5, 5.5, 8.2 and 9.0 kb bands are shown. Hybridization of this blot with a nick-translated *GAPDH* probe revealed very similar signals in each lane, indicating that approximately the same amount of total RNA was run in each lane (not shown).

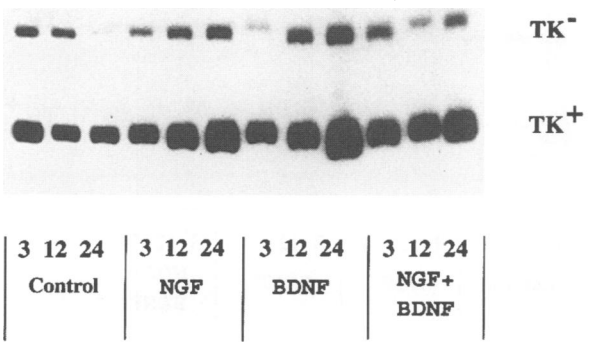

Fig. 4. Autoradiogram of a gel from a typical E11 trigeminal culture showing the amplification products of RT–PCR reactions amplified with primers specific for the 9.0 kb *trkB* transcript encoding the catalytic receptor (TK+) and with primers specific for the 8.2 kb transcript encoding a truncated, non-catalytic receptor (TK–). The RT–PCR reactions were carried out with similar levels of total RNA from cultures of E11 neurons grown for 3, 6 or 24 h with no neurotrophins (control), 5 ng/ml NGF, 5 ng/ml BDNF or NGF plus BDNF. Similar levels of RNA were used in each of these reactions as determined by separate RT–PCR reactions using primers specific for GAPDH. Very similar autoradiograms were obtained in three separate experiments.

2.5 kb transcripts, suggesting that neurons express both catalytic and non-catalytic TrkB variants.

Regulation of trkB mRNA expression

We used semi-quantitative RT–PCR to investigate the effect of neurotrophins on the expression of *trkB* mRNA in cultured trigeminal neurons using primers specific for the 9.0 kb *trkB* transcript which encodes the catalytic TrkB receptor, and primers specific for the 8.2 kb *trkB* transcript which encodes a truncated TrkB receptor. To compare the relative levels of these transcripts in neurons under different experimental conditions, we carried out PCR reactions on aliquots of reversed-transcribed RNA that contained the same amount of first-strand cDNA. The size of aliquots used was based on the results of a series of PCR reactions on the reverse-transcribed RNA using *GAPDH*-specific primers; aliquots that gave the same amounts of *GAPDH* amplification products were considered to contain the same amount of first-strand cDNA. Although this method of detecting *trkB* mRNA in small numbers of cultured neurons is not quantitative, it permits detection of differences in the level of *trkB* mRNA expression under different experimental conditions.

The expression of *trkB* mRNA was studied *in vitro* at two stages: at E11, when the expression of the 9.0 kb *trkB* transcript is increasing *in vivo*, and at E13, when the expression of this transcript is decreasing. At both ages, the level of the 8.2 kb *trkB* transcript is increasing. The neurons were cultured for 24 h under four different experimental conditions: in defined medium with no added neurotrophins (control cultures), with 5 ng/ml BDNF, with 5 ng/ml NGF and with BDNF plus NGF. RNA was extracted from these cultures at different times after plating, at 3, 6 and 24 h. In E11 cultures, the majority of neurons survived for 24 h under all conditions: virtually all neurons survived with BDNF or BDNF plus NGF, and between 70 and 85% survived in control cultures and cultures containing NGF. In E13 cultures, the number of neurons surviving in control cultures had dropped to between 20 and 30% by 24 h, between 50 and 60% of the neurons survived with BDNF and almost all of the neurons were supported by NGF or NGF plus BDNF at this time. Shortly after plating, the proportion of neurons identified in these cultures ranged between 50 and 60%. However, because the serum-free medium used in these cultures was not suitable for the survival of non-neuronal cells, the number of these cells dropped to <15% by 24 h.

In E11 cultures, similar levels of the amplification product corresponding to the 9.0 kb *trkB* transcript were obtained under all experimental conditions after 3 h incubation (Figure 4). In control cultures, there was a small decrease in *trkB* amplification product after 24 h; however, in cultures containing BDNF alone, NGF alone and BDNF plus NGF, the levels increased by a similar amount between 3 and 24 h. Densitometry of the PCR gels from three separate experiments revealed an ~5-fold higher level of amplification product in neurotrophin-supplemented cultures compared with control cultures after 24 h incubation. Although this difference does not necessarily reflect the actual difference in *trkB* mRNA levels between E11 neurons grown with and without neurotrophins, it nonetheless indicates that BDNF and NGF maintain or increase the expression of the 9.0 kb *trkB* transcript in the neurons at this stage of development.

The level of the amplification product corresponding to the 8.2 kb *trkB* transcript also decreased with time in E11 control cultures (Figure 4), although the decrease was more marked than for the amplification product for the 9.0 kb *trkB* transcript. BDNF and NGF also increased the level of the amplification product corresponding to the

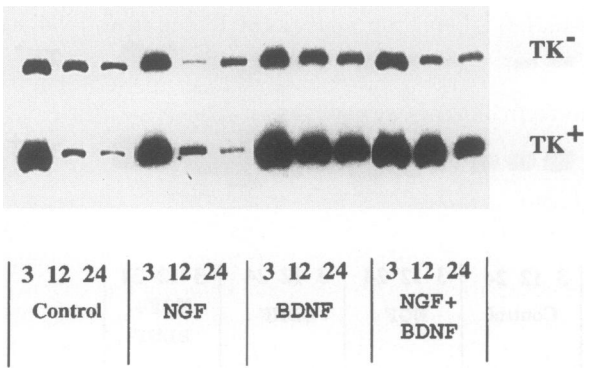

Fig. 5. Autoradiogram of a gel from a typical E13 trigeminal culture showing the amplification products of RT–PCR reactions amplified with primers specific for the 9.0 kb *trkB* transcript encoding the catalytic receptor (TK+) and with primers specific for the 8.2 kb transcript encoding a truncated, non-catalytic receptor (TK–). The RT–PCR reactions were carried out with similar levels of total RNA from cultures of E13 neurons grown for 3, 12 or 24 h with no neurotrophins (control), 5 ng/ml NGF, 5 ng/ml BDNF or NGF plus BDNF. Similar levels of RNA were used in each of these reactions as determined by separate RT–PCR reactions using primers specific for GAPDH. Very similar autoradiograms were obtained in three separate experiments.

8.2 kb *trkB* transcript between 3 and 24 h in these cultures. Similar results were obtained in three separate experiments. These findings suggest that BDNF and NGF also maintain or increase the expression of the 8.2 kb *trkB* transcript in E11 neurons.

In E13 cultures, similar levels of the amplification product corresponding to the 9.0 kb *trkB* transcript were obtained under all experimental conditions after 3 h incubation (Figure 5). In both control cultures and NGF-supplemented cultures, there was a marked fall in *trkB* amplification product between 3 and 12 h, and a further fall between 12 and 24 h. In contrast, there was little change between 3 and 12 h in the levels of *trkB* amplification product in cultures containing BDNF or BDNF plus NGF. Although, there was a small overall decrease in *trkB* amplification product between 3 and 24 h in BDNF-supplemented cultures, by 24 h densitometry of the PCR gels from three separate experiments revealed a 6- to 10-fold higher level of amplification product in BDNF-supplemented cultures compared with control cultures and NGF-supplemented cultures. These results suggest that whereas BDNF still positively affects expression of the 9.0 kb *trkB* transcript by E13, NGF no longer affects its expression by this stage.

The level of the amplification product corresponding to the 8.2 kb *trkB* transcript also decreased between 3 and 24 h in control cultures and NGF-supplemented cultures at E13 (Figure 5), although these decreases were less marked than the decreases in the amplification product for the 9.0 kb *trkB* transcript in these cultures (2- to 3-fold decreases compared with 12- to 15-fold decreases in densitometric measurements made from three separate experiments). Two- to 3-fold decreases were also observed in the amplification product corresponding to the 8.2 kb *trkB* transcript between 3 and 24 h in BDNF-supplemented cultures. These findings suggest that BDNF and NGF have little effect on the expression of the 8.2 kb *trkB* transcript by E13.

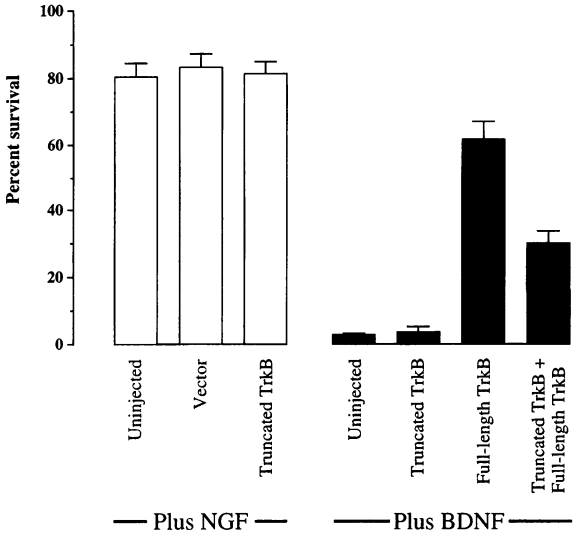

Fig. 6. Bar chart of the number of P2 SCG neurons surviving 48 h after NGF deprivation and microinjection expressed as a percentage of the number of neurons at the time of deprivation. Purified neurons were first grown overnight with NGF, washed to remove the NGF and injected (except the uninjected controls) with the pMEX vector without inserted DNA (vector), the pMEX vector containing full-length $trkB^{145}$ cDNA (full-length TrkB), the pMEX vector containing truncated $trkB^{95}$ cDNA (truncated TrkB) or both plasmids together and were grown for 48 h with either NGF or BDNF (each at 2 ng/ml). In all cases, the expression constructs were injected at a concentration of 100 μg/ml (for co-injected constructs, each was also injected at a concentration of 100 μg/ml). The bar chart shows the combined results of three separate experiments (mean and standard errors are illustrated).

Modulation of the BDNF survival response by truncated TrkB

To test directly whether the BDNF survival response of neurons expressing full-length TrkB is affected by expression of truncated TrkB, we used microinjection to introduce expression constructs for these TrkB isoforms into NGF-responsive superior cervical ganglion (SCG) sympathetic neurons of the postnatal day 2 (P2) mouse. Purified cultures of these neurons were grown overnight with NGF and were deprived of this factor by extensive washing the following day. The neurons were then injected with expression constructs and grown for a further 2 days with either NGF or BDNF. Approximately 80% of uninjected neurons that were re-supplemented with NGF survived for 48 h, whereas <5% of uninjected neurons survived with BDNF (Figure 6). Neurons that were injected either with the pMEX vector alone or with the pMEX vector containing truncated $trkB^{95}$ cDNA (2.5 kb transcript) responded normally to NGF. There was no significant difference between the number of neurons surviving with NGF after injection with these plasmids compared with the number of uninjected neurons surviving with NGF ($P > 0.1$, *t*-test). Whereas injection of the pMEX vector containing truncated $trkB^{95}$ cDNA did not confer a BDNF survival response on SCG neurons, 61% of SCG neurons survived with BDNF following injection of the pMEX vector containing full-length $trkB^{145}$ cDNA. Although injection of the pMEX vector containing truncated $trkB^{95}$ cDNA did not affect the NGF survival response, injection of this construct together with the pMEX vector containing full-length $trkB^{145}$ cDNA at a 1:1 ratio caused

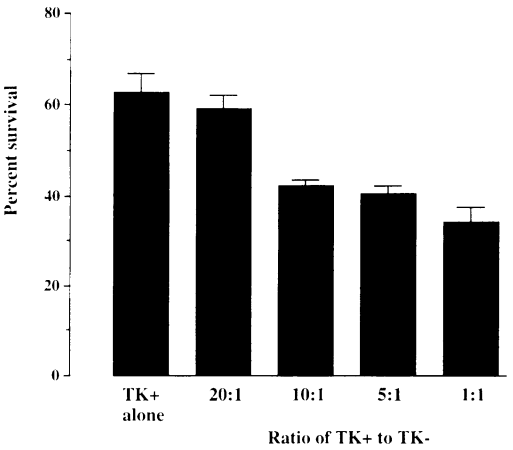

Fig. 7. Bar chart showing the percent survival of P2 SCG neurons incubated with 2 ng/ml BDNF for 48 h after microinjection with the pMEX vector containing full-length $trkB^{145}$ cDNA on its own (TK+ alone) or this vector plus the pMEX vector containing truncated $trkB^{95}$ cDNA (TK–) at different ratios (20:1, 10:1, 5:1 and 1:1). The TK+ plasmid was injected at a concentration of 100 μg/ml and the level of the TK– plasmid varied. The overall concentration of injected DNA in each case was made up to 200 μg/ml with empty pMEX vector. The bar chart shows the combined results of three separate experiments (mean and standard errors are illustrated).

a clear, 2-fold, statistically significant reduction in the BDNF survival response (from 61 to 30%, $P < 0.001$, *t*-test). These results suggest that truncated TrkB specifically down-regulates BDNF signalling via TrkB but does not affect NGF signalling via endogenously expressed TrkA.

To investigate how effective much lower ratios of truncated to full-length TrkB are in reducing the BDNF survival response, experiments were set up in which sympathetic neurons were injected with the $trkB^{95}$ and $trkB^{145}$ expression plasmids at different ratios. In these experiments, the concentration of the $trkB^{145}$ plasmid was held constant (100 μg/ml) and the concentration of the $trkB^{95}$ plasmid varied. The overall concentration of injected DNA was kept at 200 μg/ml using the empty pMEX vector. In these experiments (Figure 7), a ratio of $trkB^{95}$ to $trkB^{145}$ plasmids as low as 1:10 caused a statistically significant decrease in the number of neurons surviving with BDNF compared with neurons injected with the $trkB^{145}$ plasmid alone ($P < 0.05$, *t*-test). Although the BDNF survival response of neurons injected with a 1:20 ratio of $trkB^{95}$ to $trkB^{145}$ plasmids was less than neurons injected with the $trkB^{145}$ plasmid alone, this reduction was not statistically significant. Although the actual ratios of expressed TrkB proteins in these experiments are not known, these results suggest that low levels of truncated TrkB may be very effective negative modulators of BDNF signalling.

Discussion

Previous studies using *in situ* hybridization have shown that whereas a variety of neurons express full-length *trkB* mRNA, transcripts for truncated TrkB have been detected in various non-neuronal cells. In the adult nervous system, truncated *trkB* mRNA is expressed in ependyma, choroid plexus, pia mater, astrocytes and Schwann cells (Klein *et al.*, 1990b; Merlio *et al.*, 1992; Beck *et al.*, 1993; Frisen *et al.*, 1993; Funakoshi *et al.*, 1993; Rudge *et al.*, 1994) and, during development truncated *trkB* mRNA is expressed in several mesenchymal tissues (Biffo *et al.*, 1995). Using Northern blotting, we have shown that RNA extracted from purified preparations of embryonic trigeminal neurons contains the 9.0 kb *trkB* transcript encoding the full-length receptor and the 8.2 and 2.5 kb transcripts encoding truncated receptors that lack the catalytic tyrosine kinase domain. This finding implies that truncated TrkB receptors are not restricted to non-neuronal cells but that trigeminal neurons co-express both full-length and truncated TrkB receptors. Interestingly, transcripts for full-length and truncated TrkB recently have been shown to be expressed in overlapping populations of adult rat spinal motoneurons (Armanini *et al.*, 1995).

At E10, when the earliest trigeminal axons are growing to their targets and the neurons survive independently of neurotrophins in culture (Davies and Lumsden, 1984; Buchman and Davies, 1993), the level of *trkB* transcripts was very low. The level of *trkB* transcripts, especially the 9.0 kb transcript, increased markedly between E10 and E11, coinciding with the acquisition of BDNF dependence by E10 neurons after 24 h *in vitro* (Buchman and Davies, 1993; Paul and Davies, 1995). This suggests that the onset of BDNF dependence is controlled by the expression of functional TrkB receptors. Studies of *trkA* mRNA expression in developing trigeminal neurons likewise have shown that the subsequent onset of NGF responsiveness coincides with a marked increase in the expression of *trkA* mRNA (Wyatt and Davies, 1993).

The expression of the 9.0 kb *trkB* transcript was substantially higher than that of the 8.2 and 2.5 kb transcripts in E11 and E12 trigeminal ganglia. After a peak at E12, the level of the 9.0 kb *trkB* transcript fell, whereas the levels of the 8.2 and 2.5 kb transcripts continued to increase with age and to exceed that of the 9.0 kb transcript. The predominance of the transcript for the full-length, catalytic TrkB receptor at E11 and E12 coincides with the period of enhanced neuronal loss in the trigeminal ganglia of $trkB^{-/-}$ mice, indicating that many trigeminal neurons depend on TrkB signalling for survival during this stage of development (Piñón *et al.*, 1996). The increase in the relative levels of transcripts for truncated TrkB to the transcript for full-length TrkB after E12 takes place over the period of development when trigeminal neurons lose survival dependence on BDNF both *in vivo* (Piñón *et al.*, 1996) and *in vitro* (Buchman and Davies, 1993; Paul and Davies, 1995). The number of neurons with pyknotic nuclei in the trigeminal ganglia of $trkB^{-/-}$ mice at E11 and E12 is 3- to 4-fold higher than that in wild-type ganglia, and falls to below that in wild-type ganglia by E13 (Piñón *et al.*, 1996). Likewise, the number of neurons surviving with BDNF for 48 h *in vitro* falls from virtually all neurons in E11 cultures to <10% in E13 cultures (Buchman and Davies, 1993). Although we do not know the relationship between *trkB* mRNA and TrkB protein levels, studies of other neurotrophin receptors suggest that mRNA levels provide a reasonable indication of the expression of the respective proteins. For example, quantitative RT–PCR and quantitative Western blotting have shown a close correlation between developmental changes in the ratio of *trkA* mRNA to *p75* mRNA and the ratio of TrkA to p75 protein

in sympathetic neurons (A.Horton, G.Laramee, S.Wyatt, A.Shih, J.Winslow and A.M.Davies, unpublished data). In addition, the marked increase in *trkA* and *p75* mRNAs that occurs in trigeminal neurons shortly after their axons reach their targets (Wyatt and Davies, 1993) is correlated with the appearance of NGF binding sites on these neurons (Davies *et al.*, 1987). Thus, it is likely that the decreasing responsiveness of trigeminal neurons to BDNF after E12 is associated with an increase in the ratio of truncated TrkB receptors to full-length TrkB receptors. This raises the possibility that non-catalytic TrkB receptors function as negative regulators of BDNF signalling.

To test directly the possibility that truncated TrkB receptors modulate the BDNF survival response in neurons, we microinjected vectors expressing full-length and truncated TrkB in postnatal sympathetic neurons which do not normally respond to BDNF. Whereas full-length TrkB alone enabled the majority of these neurons to survive in response to BDNF, co-expression of truncated TrkB with full-length TrkB caused a marked reduction in the number of neurons surviving in the presence of this factor. Moreover, even very low ratios of truncated TrkB to full-length TrkB expression plasmids caused a large decrease in the BDNF survival response, suggesting that truncated TrkB is a very effective negative regulator of BDNF signalling. This negative regulatory effect of truncated TrkB was specific for the BDNF survival response because expression of truncated TrkB had no effect on the NGF survival response, which is mediated via TrkA. Previous experiments with mutated TrkA receptors that lack a kinase domain have shown that these receptors can act as suppressors of NGF signalling via wild-type TrkA receptors in cell lines (Jing *et al.*, 1992). COS cells co-expressing wild-type TrkA receptors and kinase-deficient mutated TrkA receptors had significantly fewer transformation foci in the presence of NGF compared with COS cells expressing wild-type TrkA receptors alone. It has also been demonstrated recently that ^{45}Ca efflux responses to BDNF in oocytes ectopically expressing catalytic TrkB are reduced by the co-expression of non-catalytic TrkB (Eide *et al.*, 1996).

Because BDNF signalling is dependent on phosphorylation of the kinase domains of full-length TrkB receptors, truncated TrkB receptors could act as negative regulators of BDNF signalling in neurons in at least two ways. These receptors may simply compete with full-length TrkB for BDNF binding and hence reduce the occupancy of full-length receptors. Alternatively, because each neurotrophin homodimer binds to two Trk receptors which are thought to transphosphorylate each other when their kinase domains are approximated by neurotrophin binding (Jing *et al.*, 1992), phosphorylation would fail to occur if a BDNF molecule bound to one truncated and one full-length TrkB receptor. Increasing the proportion of truncated to full-length receptors would decrease the probability of BDNF binding to two full-length receptors.

Modulation of BDNF signalling is only one of the potential functions of truncated TrkB receptors. The widespread expression of truncated TrkB by astrocytes in the adult central nervous system (CNS) (Klein *et al.*, 1990; Frisen *et al.*, 1993; Rudge *et al.*, 1994) together with the limited spread of injected BDNF in the brain parenchyma compared with NGF (Morse *et al.*, 1993) raises the possibility that truncated TrkB may play an important role in limiting the diffusion of BDNF in the mature CNS. The demonstration that truncated TrkB receptors on leptomeningeal cells bind BDNF with high affinity and rapidly internalize bound BDNF (Biffo *et al.*, 1995) suggests that selective uptake limits the spread of BDNF from its sites of synthesis in the CNS. In the chicken embryo, truncated TrkB is expressed early by non-neuronal cells that abut regions of BDNF synthesis, suggesting that in these locations truncated TrkB may form a boundary that restricts the diffusion of BDNF. For example, truncated TrkB is expressed by the mesenchymal cells that surround the otic vesicle which contains cells that express BDNF mRNA (Biffo *et al.*, 1995). A similar role was suggested previously for p75 in restricting the diffusion of NGF in developing skin because p75 is expressed by the mesenchymal cells (Wyatt *et al.*, 1990) that lie beneath cutaneous epithelium which expresses high levels of NGF (Davies *et al.*, 1987).

Cross-linking of iodinated BDNF to TrkB in protein extracts of various regions of the developing CNS has shown that the expression of full-length TrkB precedes the expression of truncated TrkB in embryonic and postnatal development (Allendoerfer *et al.*, 1994; Escandon *et al.*, 1994). Although the functional significance of this switch from full-length to truncated TrkB expression in these brain regions is unclear, there is a decrease in BDNF-induced TrkB tyrosine phosphorylation in cortical tissue as the ratio of truncated to full-length TrkB increases (Knusel *et al.*, 1994), raising the possiblity that truncated TrkB may play a role in regulating BDNF responses in this tissue.

Our *in vitro* studies have shown that the expression of the 9.0 and 8.2 kb *trkB* transcripts, which encode full-length and truncated receptors respectively, is regulated differently by NGF and BDNF during development. At E11, when the levels of the 9.0 and 8.2 kb *trkB* mRNAs are increasing *in vivo*, the levels of these mRNAs in cultured neurons also increase in the presence of NGF and BDNF but decrease in the absence of neurotrophins. At E13, when the level of the 9.0 kb *trkB* mRNA has started to decrease *in vivo*, the level of this mRNA in cultured neurons decreases markedly in both control cultures and cultures containing NGF. The level of the 8.2 kb mRNA, which is still increasing at E13 *in vivo*, decreases slowly under all *in vitro* conditions and there are similar levels of this mRNA expressed in the presence and absence of neurotrophins after 24 h *in vitro*.

It is likely that at least a proportion of trigeminal neurons are exposed to both BDNF and NGF as early as E11 *in vivo*. BDNF mRNA is first detected in the maxillary process, the most densely innervated part of the trigeminal territory, at E10 (Buchman and Davies, 1993), and NGF mRNA and protein are detected at E11 (Davies *et al.*, 1987). Thus, the developmental changes in the effects of NGF and BDNF on the expression of the 9.0 kb *trkB* mRNA seen *in vitro* correlate with the changing expression of this transcript observed *in vivo*. Both NGF and BDNF up-regulate this mRNA *in vitro* at the stage when this is increasing *in vivo*, and its level falls in cultures supplemented with NGF and BDNF at the time when this is falling *in vivo*. Although the level of the 9.0 kb *trkB* mRNA in E13 cultures falls less abruptly in the presence

of BDNF compared with NGF, the level of BDNF mRNA in the trigeminal territory is much lower than NGF mRNA at E13 (Buchman and Davies, 1993). This, combined with the marked decrease in BDNF responsiveness of trigeminal neurons with age (Buchman and Davies, 1993; Buj-Bello *et al.*, 1994), suggests that endogenous BDNF is unlikely to have a major influence on *trkB* mRNA expression in trigeminal neurons at E13 *in vivo*. The up-regulation of the 8.2 kb mRNA by BDNF and NGF in E11 cultures is also consistent with BDNF and NGF up-regulating this transcript at this stage *in vivo*. However, by E13, BDNF and NGF have no obvious effect on the expression of this mRNA *in vitro*, suggesting that other factors may be involved in up-regulating the expression of this transcript at later embryonic stages.

The effects of BDNF and NGF on *trkB* and *trkA* mRNA expression in developing trigeminal neurons differ in several ways. Whereas BDNF down-regulates *trkA* mRNA expression in E11 trigeminal neurons in culture, NGF does not affect *trkA* mRNA expression at either this stage or later stages *in vitro* (Wyatt and Davies, 1993). Furthermore, *trkA* mRNA increases normally between E11 and E13 in the trigeminal ganglia of embryos that are homozygous for a null mutation in the NGF gene (Davies *et al.*, 1995), indicating that *trkA* expression is regulated independently of NGF in developing sensory neurons. Likewise, *trkA* mRNA is regulated independently of NGF in developing sympathetic neurons (Wyatt and Davies, 1995). Whether the effects we have observed of NGF and BDNF on *trkB* mRNA expression in cultured trigeminal neurons are physiologically relevant will have to await analysis of *trkB* mRNA expression in the neurons of embryos that have targeted mutations in the BDNF gene. The differential effects of BDNF and NGF on the expression of *trkB* and *trkA* mRNA in cultured neurons indicate that the downstream signalling events from TrkA and TrkB must differ in certain respects, as activation of these receptors has different effects on *trk* gene expression. This is surprising as the kinase domains of TrkA and TrkB have very similar structures (Klein *et al.*, 1989; Martin-Zanca *et al.*, 1989).

In summary, we have presented direct evidence for a novel role for tyrosine kinase-deficient neurotrophin receptors in the developing nervous system. We have correlated the loss of a physiologically relevant BDNF survival response with an increase in the expression of transcripts encoding non-catalytic TrkB receptors in developing trigeminal neurons, and have shown directly in expression studies in neurons that these non-catalytic TrkB receptors act as negative regulators of the BDNF survival response. Non-catalytic isoforms have also been described for TrkC, the preferred receptor for NT3 (Lamballe *et al.*, 1993; Okazawa *et al.*, 1993; Tsoulfas *et al.*, 1993; Valenzuela *et al.*, 1993). Because TrkC is expressed persistently in many trigeminal neurons beyond the period when they respond to NT3 (Ernfors *et al.*, 1992), it will be interesting to determine whether non-catalytic TrkC receptors function in an analogous way to negative regulators of NT3 signalling during development.

Materials and methods

Neuron cultures

Cultures of embryonic mouse trigeminal ganglion neurons were used to study the regulation of *trkB* mRNA expression. Embryos were obtained from overnight matings of CD1 mice. Pregnant females were killed by cervical dislocation and the stage of embryos was determined by the criteria of Theiler (1972). Dissected trigeminal ganglia were incubated for 8 min at 37°C with 0.05% trypsin (Worthington) in calcium- and magnesium-free Hank's balanced salt solution, washed with Ham's F12 medium containing 10% heat-inactivated horse serum and were triturated gently with a fire-polished, siliconized Pasteur pipette to give a single cell suspension. The cells were plated at a density of 1000–1500 neurons per dish in 35 mm plastic tissue culture dishes (Nunc) that had been pre-coated with polyornithine (0.5 mg/ml, overnight) and laminin (20 μg/ml for 4 h). The neurons were incubated at 37°C in a humidified 4.5% CO_2 incubator in a defined medium consisting of Ham's F14 supplemented with 2 mM glutamine, 0.35% bovine serum albumin (Pathocyte-4, ICN), 60 ng/ml progesterone, 16 μg/ml putrescine, 400 ng/ml L-thyroxine, 38 ng/ml sodium selenite, 340 ng/ml triiodothyronine, 60 μg/ml penicillin and 100 μg/ml streptomycin. The neurons were grown with purified recombinant NGF or BDNF (gifts of John Winslow and Gene Burdon, Genentech, Inc.).

Cultures of NGF-dependent sympathetic neurons from the SCG of P2 mice were used to study the role of truncated TrkB in modulating BDNF signalling via catalytic TrkB. The ganglia were incubated for 10 min with 0.1% trypsin and were dissociated into a single cell suspension by gentle trituration. The neurons were separated from non-neuronal cells by differential sedimentation at 1 *g* (Davies, 1986) and were plated in 60 mm polyornithine/laminin-coated plastic Petri dishes in defined culture medium (above).

Northern blotting

Northern blotting was used to assess the relative levels of *trkB* transcripts in trigeminal ganglia at different stages of development or in preparations (>95% pure) of trigeminal neurons separated from the non-neuronal cells in the ganglion by low temperature differential sedimentation (Davies, 1986). RNA extraction using guanidinium isothiocyanate electrophoresis in 1.2% agarose–formaldehyde gels and blotting to Hybond-N filters (Amersham) were done as described previously (Buchman and Davies, 1993; Buchman *et al.*, 1994). The filters were hybridized with a 0.5 kb ^{32}P-labelled nick-translated probe made from the extracellular domain of mouse *trk*B cDNA (spanning 644–1120 bp) (Klein *et al.*, 1989). Hybridization was carried out for 48 h at 42°C in 50% formamide, 5× SSC, 50 mM sodium phosphate pH 7.0, 5 mM EDTA, 0.5% SDS, 5× Denhardt's solution, 250 μg/ml salmon sperm DNA and 50 μg/ml *Escherichia coli* tRNA. The filters were washed twice in 0.2× SSC with 0.2% SDS at 68°C before exposure to X-ray film. To compare the relative levels of RNA, the filters were rehybridized with a ^{32}P-labelled nick-translated mouse GAPDH probe under the same conditions. Films were scanned using a Molecular Dynamic densitometer.

RT–PCR

RT–PCR was used to compare the level of the 9.0 kb (TK+) *trk*B transcript at intervals in trigeminal neurons cultured under different experimental conditions. Total RNA was extracted from these cultures using guanidinium isothiocyanate and was purified using easiRNA (Nuncleon kit, Scotlab) after treatment with DNase I. Purified RNA was reverse transcribed with hexamer random primers (0.1 μg of random primers per 1 μg of total RNA) using Superscript reverse transcriptase (Gibco BRL).

PCR was first carried out using primers for *GAPDH* to equalize the starting amounts of first strand cDNA in different experimental samples for PCR with *trkB* primers. The *GAPDH* primers were: 5′-CTTCATTGACCTCAACTACATG (forward primer) and 5′-ATGGCATGGACTGTGGTCAT (reverse primer). The primers specific for the 9.0 kb full-length *trkB* transcript were: 5′-CAGTATTAACTCGCTTCTGGC (forward primer) and 5′-TTCATCCACGTCAAAGGCAG (reverse primer). The primers specific for the 8.2 kb truncated *trkB* transcript were: 5′-GTCATAGCTAGGTCTAAGTGC (forward primer) and 5′-GGCAATGGAAAGGGACAAGA (reverse primer). The primers were end-labelled with [^{32}P]ATP and were purified using easiMER (Nuncleon kit, Scotlab). PCR was carried out for 18–25 cycles: 95°C for 45 s, 58°C for 30 s and 72°C for 60 s. The amplified products were separated on 6% acrylamide gels that were autoradiographed using X-ray film after drying.

The specificity of the primers for the 9.0 kb *trkB* transcript was confirmed by using a ^{32}P-labelled DNA probe derived from the 150 bp cDNA fragment amplified by these primers to probe Northern blots of adult brain total RNA. This probe hybridized to a single 9.0 kb band (not shown). The specificity of the primers for the 8.2 kb *trkB* transcript was confirmed in a similar manner.

Microinjection of TrkB expression constructs

P2 SCG neurons were grown initially for 12 h in defined medium containing 2 ng/ml NGF. After this time, the cultures were washed extensively with neurotrophin-free medium and were pressure-injected (Allsopp *et al.*, 1993) with the pMEX vector containing full-length $trkB^{145}$ cDNA, the pMEX vector containing truncated $trkB^{95}$ cDNA (2.5 kb transcript) or both constructs together (Klein *et al.*, 1989). Unless otherwise stated, each construct was injected at a concentration of 100 μg/ml in phosphate-buffered saline (PBS). After injection, the culture medium was supplemented with either 2 ng/ml NGF or 2 ng/ml BDNF and the number of neurons surviving in each experimental condition was counted 48 h later and expressed as a percentage of the number of neurons injected.

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References

Allendoerfer,K.L., Cabelli,R.J., Escandon,E., Kaplan,D.R., Nikolics,K. and Shatz,C.J. (1994) Regulation of neurotrophin receptors during the maturation of the mammalian visual system. *J. Neurosci.*, **14**,1795–1811.

Allsopp,T.E., Wyatt,S., Paterson,H.F. and Davies,A.M. (1993) The proto-oncogene *bcl-2* can selectively rescue neurotrophic factor-dependent neurons from apoptosis. *Cell*, **73**, 295–307.

Armanini,M.P., McMahon,S.B., Sutherland,J., Shelton,D.L. and Phillips,H.S. (1995) Truncated and catalytic isoforms of trkB are co-expressed in neurons of rat and mouse CNS. *Eur. J. Neurosci.*, **7**, 1403–1409.

Arumae,U., Pirvola,U., Palgi,J., Kiema,T.R., Palm,K., Moshnyakov,M., Ylikoski,J. and Saarma,M. (1993) Neurotrophins and their receptors in rat peripheral trigeminal system during maxillary nerve growth. *J. Cell Biol.*, **122**, 1053–1065.

Barbacid,M. (1994) The Trk family of neurotrophin receptors. *J. Neurobiol.*, **25**, 1386–1403.

Beck,K.D., Lamballe,F., Klein,R., Barbacid,M., Schauwecker,P.E., McNeill,T.H., Finch,C.E., Hefti,F. and Day,J.R. (1993) Induction of noncatalytic TrkB neurotrophin receptors during axonal sprouting in the adult hippocampus. *J. Neurosci.*, **13**, 4001–4014.

Biffo,S., Offenhauser,N., Carter,B.D. and Barde,Y.A. (1995) Selective binding and internalisation by truncated receptors restrict the availability of BDNF during development. *Development*, **121**, 2461–2470.

Buchman,V.L. and Davies,A.M. (1993) Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. *Development*, **118**, 989–1001.

Buchman,V.L., Sporn,M. and Davies,A.M. (1994) Role of transforming growth factor-beta isoforms in regulating the expression of nerve growth factor and neurotrophin-3 mRNA levels in embryonic cutaneous cells at different stages of development. *Development*, **120**, 1621–1629.

Buj-Bello,A., Pinon,L.G. and Davies,A.M. (1994) The survival of NGF-dependent but not BDNF-dependent cranial sensory neurons is promoted by several different neurotrophins early in their development. *Development*, **120**, 1573–1580.

Conover,J.C. *et al.* (1995) Neuronal deficits, not involving motor neurons, in mice lacking BDNF and NT4. *Nature*, **375**, 235–238.

Davies,A.M. (1986) The survival and growth of embryonic proprioceptive neurons is promoted by a factor present in skeletal muscle. *Dev. Biol.*, **115**, 56–67.

Davies,A.M. (1994) Role of neurotrophins in the developing nervous system. *J. Neurobiol.*, **25**, 1334–1348.

Davies,A.M. and Lumsden,A.G.S. (1984) Relation of target encounter and neuronal death to nerve growth factor responsiveness in the developing mouse trigeminal ganglion. *J. Comp. Neurol.*, **223**, 124–137.

Davies,A.M., Bandtlow,C., Heumann,R., Korsching,S., Rohrer,H. and Thoenen,H. (1987) Timing and site of nerve growth factor synthesis in developing skin in relation to innervation and expression of the receptor. *Nature*, **326**, 353–358.

Davies,A.M., Horton,A., Burton,L.E., Schmelzer,C., Vandlen,R. and Rosenthal,A. (1993) Neurotrophin-4/5 is a mammalian-specific survival factor for distinct populations of sensory neurons. *J. Neurosci.*, **13**, 4961–4967.

Davies,A.M., Wyatt,S., Nishimura,M. and Phillips,H. (1995) NGF receptor expression in sensory neurons develops normally in embryos lacking NGF. *Dev. Biol.*, **171**, 434–438.

Eide,F.F., Vining,E.R., Eide,B.L., Zang,K., Wang,X.Y. and Reichardt,L.F. (1996) Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signalling. *J. Neurosci.*, **16**, 3123–3129.

Ernfors,P., Merlio,J.-P. and Persson,H. (1992) Cells expressing mRNA for neurotrophins and their receptors during embryonic rat development. *Eur. J. Neurosci.*, **4**, 1140–1158.

Ernfors,P., Lee,K.F. and Jaenisch,R. (1994) Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature*, **368**, 147–150.

Escandon,E., Soppet,D., Rosenthal,A., Mendoza,R.J., Szonyi,E., Burton,L.E., Henderson,C.E., Parada,L.F. and Nikolics,K. (1994) Regulation of neurotrophin receptor expression during embryonic and postnatal development. *J. Neurosci.*, **14**, 2054–2068.

Frisen,J., Verge,V.M., Fried,K., Risling,M., Persson,H., Trotter,J. and Lindholm,D. (1993) Characterisation of glia trkB receptors: differential response to injury in the central and peripheral nervous systems. *Proc. Natl Acad. Sci. USA*, **90**, 4971–4975.

Funakoshi,H., Frisen,J., Barbany,G., Timmusk,T., Zachrisson,O., Verge,V.M. and Persson,H. (1993) Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *J. Cell Biol.*, **123**, 455–465.

Jing,S., Tapley,P. and Barbacid,M. (1992) Nerve growth factor mediates signal transduction through trk homodimer receptors. *Neuron*, **9**, 1067–1079.

Jones,K.R., Farinas,I., Backus,C. and Reichardt,L.F. (1994) Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell*, **76**, 989–999.

Kaplan,D.R. and Stephens,R.M. (1994) Neurotrophin signal transduction by the Trk receptor. *J. Neurobiol.*, **25**, 1404–1417.

Klein,R. (1994) Role of neurotrophins in mouse neuronal development. *FASEB J.*, **8**, 738–744.

Klein,R., Parada,L.F., Coulier,F. and Barbacid,M. (1989) trkB, a novel tyrosine protein kinase receptor expressed during mouse neural development. *EMBO J.*, **8**, 3701–3709.

Klein,R., Conway,D., Parada,L.F. and Barbacid,M. (1990a) The *trkB* tyrosine protein kinase gene codes for a second neurogenic receptor that lacks the catalytic kinase domain. *Cell*, **61**, 647–656.

Klein,R., Martin,Z.D., Barbacid,M. and Parada,L.F. (1990b) Expression of the tyrosine kinase receptor gene *trkB* is confined to the murine embryonic and adult nervous system. *Development*, **109**, 845–50.

Knusel,B., Rabin,S.J., Hefti,F. and Kaplan,D.R. (1994) Regulated neurotrophin responsiveness during neuronal migration and early differentiation. *J. Neurosci.*, **14**, 1542–1554.

Lamballe,F., Tapley,P. and Barbacid,M. (1993) trkC encodes multiple neurotrophin-3 receptors with distinct biological properties and substrate specificities. *EMBO J.*, **12**, 3083–3094.

Liu,X., Ernfors,P., Wu,H. and Jaenisch,R. (1995) Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. *Nature*, **375**, 238–241.

Martin-Zanca,D., Oskam,R., Mitra,C., Copeland,T. and Barbacid,M. (1989) Molecular and biochemical characterisation of the human *trk* proto-oncogene. *Mol. Cell. Biol.*, **9**, 24–33.

Merlio,J.P., Ernfors,P., Jaber,M. and Persson,H. (1992) Molecular cloning of rat *trk*C and distribution of cells expressing messenger RNAs for members of the *trk* family in the rat central nervous system. *Neuroscience*, **51**, 513–532.

Morse,J.K. *et al.* (1993) Brain-derived neurotrophic factor (BDNF) prevents the degeneration of medial septal cholinergic neurons following fimbria transection. *J. Neurosci.*, **13**, 4146–4156.

Okazawa,H., Kamei,M. and Kanazawa,I. (1993) Molecular cloning and expression of a novel truncated form of chicken trkC. *FEBS Lett.*, **329**, 171–177.

Paul,G. and Davies,A.M. (1995) Trigeminal sensory neurons require extrinsic signals to switch neurotrophin dependence during the early stages of target field innervation. *Dev. Biol.*, **171**, 590–605.

Piñón,L.G.P., Minichiello,L., Klein,R. and Davies,A.M. (1996) Timing of neuronal death in *trkA*, *trkB* and *trkC* mutant embryos demonstrates neurotrophin switching in developing trigeminal neurons. *Development*, **122**, 3255–3261.

Rudge,J.S., Li,Y., Pasnikowski,E.M., Mattsson,K., Pan,L., Yancopoulos,G., Wiegand,S.J., Lindsay,R.M. and Ip,N.Y. (1994) Neurotrophic factor receptors and their signal transduction capabilities in rat astrocytes. *Eur. J. Neurosci.*, **6**, 693–705.

Theiler,K. (1972) Development and normal stages from fertilisation to 4 weeks of age. In *The House Mouse.* Springer-Verlag, Berlin, Germany, pp. 1–168.

Tsoulfas,P., Soppet,D., Escandon,E., Tessarollo,L., Mendoza,R.J., Rosenthal,A., Nikolics,K. and Parada,L.F. (1993) The rat *trkC* locus encodes multiple neurogenic receptors that exhibit differential response to neurotrophin-3 in PC12 cells. *Neuron*, **10**, 975–990.

Valenzuela,D.M. *et al.* (1993) Alternative forms of rat TrkC with different functional capabilities. *Neuron*, **10**, 963–974.

Vogel,K.S. and Davies,A.M. (1991) The duration of neurotrophic factor independence in early sensory neurons is matched to the time course of target field innervation. *Neuron*, **7**, 819–830.

Vogel,K.S. and Davies,A.M. (1993) Heterotropic transplantation of presumptive placodal ectoderm changes the fate of sensory neuron precursors. *Development*, **119**, 263–276.

Wyatt,S. and Davies,A.M. (1993) Regulation of expression of mRNAs encoding the nerve growth factor receptors p75 and trkA in developing sensory neurons. *Development*, **119**, 635–648.

Wyatt,S. and Davies,A.M. (1995) Regulation of expression of p75 and *trk*A mRNAs in developing sympathetic neurons. *J. Cell Biol.*, **130**, 1435–1446.

Wyatt,S., Shooter,E.M. and Davies,A.M. (1990) Expression of the NGF receptor gene in sensory neurons and their cutaneous targets prior to and during innervation. *Neuron*, **4**, 421–427.

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