The neurotrophin family of growth factors includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). Each member of the neurotrophin family promotes the survival of distinct, but partially overlapping, populations of developing neurons. For example, NGF is required for survival of neural crest-derived sensory and sympathetic neurons (Levi-Montalcini and Hamburger, 1953; Gorin and Johnson, 1979) and basal forebrain cholinergic neurons (Hefti, 1986; Sofroniew et al., 1990). BDNF promotes survival in dorsal root (Hofer and Barde, 1988) and nodose ganglia (Lindsay et al., 1985), retinal ganglion cells (Johnson et al., 1986), basal forebrain cholinergic cells (Phillips et al., 1990; Alderson et al., 1990), and spinal and cranial motoneurons (Oppenheim et al., 1992; Sendtner et al., 1992; Yan et al., 1992). NT-3 has trophic effects on peripheral sensory and autonomic ganglia (Maisonpierre et al., 1990). NT-4, like BDNF, has trophic effects on brainstem (Koliatsos et al., 1994) and spinal cord (Oppenheim et al., 1993b) motoneurons, as well as some populations of sensory neurons (Ibanez et al., 1993).

Neurotrophins display high- (k_d=10^{-11} M) and low- (k_d=10^{-9} M) affinity binding characteristics (see Chao, 1992; Meakin and Shooter, 1992; Barbacid, 1993; Bothwell, 1995 for reviews) and all of the neurotrophins bind with low affinity to p75, while high-affinity binding is mediated by the tropomyosin related kinase (trk) family of receptor tyrosine kinases. In general, NGF binds trkA, BDNF and NT-4 bind trkB, and NT-3 binds trkC; however, there is some overlap in receptor specificity between members of the neurotrophin family. The role of p75 in high-affinity neurotrophin binding is controversial. In some cell types neurotrophin binding to trk receptors has no biological effect in the absence of p75 (Hempstead et al., 1991; Verge et al., 1992), whereas in other cells the trk receptors can work alone (Klein et al., 1991; Radeke and Feinstein, 1991; Holtzman et al., 1992). p75 may modulate trk function or it may have some function independent of the high affinity receptors (Meakin and Shooter, 1992; Rabizadah et al., 1992; Lee et al., 1992; Dubrowsky et al., 1994).

In the chick embryo, approximately 50% of spinal cord motoneurons die during the second week of development (Hamburger, 1975). Considerable evidence suggests that...
muscle, the peripheral target of motoneurons, provides trophic molecules that are required for cell survival (Hamburger, 1934; Hollyday and Hamburger, 1976; Hamburger and Oppenheim, 1982; Oppenheim et al., 1988). For example, partially purified protein extracts from skeletal muscle increase motoneuron survival in vivo (Oppenheim et al., 1988) and in vitro (Dohrmann et al., 1986; 1987; Bloch-Gallego et al., 1991). One approach to identifying the muscle-derived factors has been to treat embryos or cultured embryonic motoneurons with purified growth factors and assay for survival. A variety of agents capable of promoting motoneuron survival have been identified in this way including platelet-derived growth factor, neurotrophin-4, transforming growth factor-β, cholineric differentiation factor (Oppenheim et al., 1993a), ciliary neurotrophic factor (Oppenheim et al., 1991), the insulin-like growth factors (Neff et al., 1993; Oppenheim et al., 1994), s100-β (Battacharyya et al., 1992), glial cell line-derived neurotrophic factor (Newsome et al., 1994; Oppenheim et al., 1995), and brain-derived neurotrophic factor (Oppenheim et al., 1992; Henderson et al., 1993; Hughes et al., 1993). However, because there are other cellular sources of trophic support for developing motoneurons, e.g., afferents and glial (Dohmann et al., 1987; Eagleson et al., 1985; Okado and Oppenheim 1984; Brenneman et al., 1987; Pollack et al., 1981), the survival assay alone does not conclusively demonstrate that a trophic factor is muscle-derived.

BDNF promotes the survival of developing and axotomized spinal cord and cranial motoneurons in the rat, chick and mouse in vivo (Oppenheim et al., 1992; 1993b; Sendtner et al., 1992; Yan et al., 1992; Koliatsos et al., 1993; 1994; Li et al., 1994; Houenou et al., 1994). There is some direct evidence that BDNF is a muscle-derived trophic factor for motoneurons. First, BDNF mRNA can be detected in the developing limb during the period of motoneuron cell death (Hallbook et al., 1993; Henderson et al., 1993; McKay et al., 1993). Second, 125I-BDNF injected into the limb is retrogradely transported by motoneurons (McKay et al., unpublished data). Finally, when the high affinity BDNF receptor, trkB, is eliminated in transgenic mice there is a reduction in the number of motoneurons present at birth (Klein et al., 1993). However, there is conflicting evidence from both in vitro and genetic studies of BDNF. In vitro, chick motoneurons do not respond to BDNF (Arakawa et al., 1990), although rat motoneurons do (Henderson et al., 1993). Furthermore, BDNF knockouts show no change in motoneuron survival (Ernfors et al., 1994; Jones et al., 1994). Thus, the role of BDNF in motoneuron development is still not clear.

The inability of BDNF to promote the survival of chick motoneurons in vitro suggests that it has an indirect effect on cell survival. For example, the neuromuscular blocking agent, curare, promotes motoneuron survival by increasing access to muscle sources of trophic support, through increased axonal branching and synaptogenesis (Pittman and Oppenheim, 1979; Dahm and Landmesser, 1988; Oppenheim, 1989; Houenou et al., 1990; 1991; Landmesser, 1992). If BDNF acts through a similar mechanism then one might expect to see increased branching of motor axons in BDNF-treated animals. This hypothesis has been tested in the present paper. An alternative explanation for the absence of an effect in vitro is that BDNF acts, in vivo, by enhancing the development of some other cell type, which in turn promotes motoneuron survival. If this is true then motoneurons might not be expected to possess the receptors for BDNF. Yet chick motoneurons have been shown to express p75 (Heuer et al., 1990a;b; Ernfors et al., 1988) and trkB (Dechant et al., 1993) at some stages of development. The studies described here include an examination of p75 and trkB expression throughout the period of cell death. The observed temporal pattern of receptor expression helps to explain many of the conflicting results for BDNF described above.

MATERIALS AND METHODS

Preparing histological sections for in situ hybridization and immunohistochemistry

Chick eggs were placed in a 37°C incubator for 2-14 days. Embryos were removed from the eggs at the desired stages of development and fixed for 4-16 hours in 4% paraformaldehyde in PBS at 4°C. After fixation the embryos were stored overnight at 4°C in 20% sucrose and then embedded in Tissue-Tek and frozen. 15 μm frozen sections were cut using a cryostat. The sections were mounted on poly-lysine coated slides and stored at ~70°C. Sections were air dried for 2-3 hours before use.

p75 immunohistochemistry

A monoclonal antibody against the low affinity NGF receptor, p75, was provided by M. Bothwell (see von Bartheld et al., 1995). The tissue sections were treated with 0.015% H2O2 in PBS with 40% methanol to quench endogenous peroxidase activity. Non-specific antibody binding was blocked with 2% BSA and normal horse serum (negative control). The culture supernatant containing the p75 antibody was applied to the sections undiluted for a period ranging from 12 to 18 hours at room temperature. The antibody was localized using an anti-mouse secondary antibody conjugated with alkaline phosphatase. The alkaline phosphatase was visualized with 4-chloronapthol and H2O2.

In situ hybridization

Cellular mRNA for p75 and trkB were detected with digoxigenin-labelled RNA probes using a modification of the method described by Schaeren-Wiemers and Gerfin-Moser (1993). Both probes recognize region coding for the extracellular domains of the endogenous mRNAs. The probes were generated by transcribing linearized plasmids containing the clones for the desired gene. The p75-containing plasmid (pGEM4Z), provided by Dr M. Bothwell (see Heuer et al., 1990a), was linearized with EcoRI and transcribing with T3 to generate anti-sense probes. Sense probes were made by linearizing with BamHI linearized plasmid with SP6 RNA polymerase. A 1.2 kb chicken trkB clone in Bluescript SK* (Garner et al., unpublished data) was linearized with EcoRI and transcribed with T3 to generate anti-sense probes. Sense probes were made by linearizing with BamHI and transcribing with T7. The sense and anti-sense trkB probes were reduced from 1.2 kb to approximately 300 bases by alkaline hydrolysis at 65°C for 10 minutes.

Sections were prehybridized overnight at room temperature in a solution containing 50% formamide, 5× SSC, 5× Denhardt’s solution, 250 μg/ml tRNA, 50 μg/ml sheared herring sperm DNA. Probes for hybridization were suspended in the same solution and applied to sections under sealed coverslips. Hybridization was carried out for 12-18 hours at 55°C (p75 probes) and 65°C (trkB probes). Sections were washed in 0.2× SSC at hybridization temperatures for 1 hour. Digoxigenin-labeled nucleotides were detected with an alkaline phosphatase conjugated anti-digoxigenin antibody. The antibody was localized with nitroblue tetrazolium and X-Phos. Addition of 0.24 mg/ml levamisole to the reaction mixture inhibited endogenous alkaline phosphatase activity.
BDNF treatment and embryonic surgery

Five μg of either BDNF or bovine serum albumin (BSA) dissolved in PBS were applied to the chorioallantoic membrane of normal chick embryos once daily from embryonic day (E)5 through E7, E6-E9, E8-E9, or E8-E11. Both BDNF and BSA were applied in 50 μl volumes. Spinal cords were fixed in Carnoy’s solution overnight, rinsed in 70% ethanol, dehydrated, cleared in xylene, embedded in paraffin, cut into 8 μm serial sections, and stained with thionin. Lateral motor column motoneurons, identified by their large cell size, large pale nuclei, and distinct nucleoli, were counted in every tenth section on both sides of the spinal cord in order to assess the effect of BDNF treatment on motoneurons (Clark and Oppenheim, 1995).

Hindlimbs were removed unilaterally on E2 (stage 17) by exposing the embryos through a hole in the shell, placing strips of neutral red-agar over the embryo for 5-10 minutes to aid visualization, and then gently removing the limb bud with fine tungsten needles. The embryos were allowed to continue development until E4.5, when treatment was begun. Embryos were treated with BDNF or BSA from E4.5 through E6.5 and killed on E7.5. Spinal cords were processed as described above and motoneurons counted to assess the effect of BDNF treatment on cells subjected to target removal.

Motility studies

Embryos treated with BDNF or BSA were observed daily (E6 through E9) under a dissecting microscope through a hole in the egg shell according to the method described by Oppenheim and Reitzel (1975). Limb movements were observed for 3-minute periods just prior to, and 30-60 minutes after, the daily BDNF treatment. Whole body movements of the embryos were excluded.

Intramuscular nerve branching

E10 embryos treated with BDNF or BSA were incubated with the monoclonal antibody C2 (provided by Dr. L. Landmesser) according to the method described by Dahm and Landmesser (1988). Briefly, after incubation with the primary antibody, the antigen was localized and visualized with a rhodamine conjugated anti-mouse secondary antibody. Whole mounts of sartorius, iliofibularis and iliotibialis muscles were prepared and viewed under ultraviolet light on a Zeiss microscope. Secondary (side) branches from the main nerve trunks were counted along 1 mm lengths as described by Dahm and Landmesser (1988).

RESULTS

p75 and trkB expression in the neuromuscular system during the period of naturally occurring cell death

Patterns of p75 and trkB expression were studied using immunohistochemistry (p75 only) and in situ hybridization (p75 and trkB). Fig. 2 shows the results of in situ hybridization and immunohistochemical localization of p75 mRNA and protein. In a developmental series, p75 mRNA is observed at high levels in the lumbar motor column throughout the cell death period (Fig. 2A-D). p75 mRNA is relatively low in the motor column, along the spinal nerves, and in the developing kidney (Fig. 1C). A similar pattern persists in motoneurons and their axons from E5 (Fig. 1B) through E12 (not shown). In addition, there is strong immunoreactivity in the dermamyotome and among cells along the myoblast migratory pathway (Figs 1B, 2G).

p75 and trkB expression in the early developing embryo

Fig. 1 shows the results of a survey of p75 IR in the early spinal cord. On E2, p75 IR is present in the ventral spinal cord (but not in the dorsal spinal cord or floor plate) in a pattern that resembles a gradient of expression (Fig. 1A). When the first motoneuron axons extend toward the limb bud on E3, p75 IR is present in the lateral

![Image](attachment:image.png)

Fig. 1. p75 immunoreactivity in the early developing spinal cord. (A) E2 spinal cord (20x). Note the IR in the ventral portion (v) of the developing spinal cord. (B) E5 spinal cord (10x). (C) E3 spinal nerve (20x). (D) E5 spinal cord (20x); negative control incubated with normal serum. d, dorsal; v, ventral; fp, floor plate; lmc, lateral motor column; n, spinal nerve; k, kidney; drg, dorsal root ganglion; ec, enteric neural crest; c, central canal; pm, perinotochordal mesenchyme. Arrowheads indicate a stream of p75 IR in the limb bud (l) that appears to be continuous with IR in the dermamyotome (dm). Scale bars, 25 μm.
Fig. 2. Localization of p75 mRNA and protein in the developing chick embryo. (A) p75 mRNA is present in the dorsal root ganglia at E4 (A), E6 (B), E8 (C), and E10 (D). In the spinal cord, p75 mRNA is present beginning on E8. Arrowheads in A-D show expression in the mesenchyme surrounding the spinal cord and in the meninges (D). E shows a high magnification of E8 dorsal root ganglia demonstrating that a subpopulation of these cells expresses trkB. F shows expression in the submucosal layer. G shows expression in the submucosal layer and the muscularis layer at E6. H is a sense control of an E6 spinal cord. Scale bar, 100 μm.

Fig. 3. Localization of trkB mRNA in the developing chick embryo. trkB mRNA is present in the dorsal root ganglia at E4 (A), E6 (B), E8 (C), and E10 (D). In the spinal cord, trkB mRNA is present beginning on E8. Arrowheads in A-D show expression in the mesenchyme surrounding the spinal cord and in the meninges (D). E shows a high magnification of E8 dorsal root ganglia demonstrating that a subpopulation of these cells expresses trkB. F shows expression in the submucosal layer. G shows expression in the submucosal layer and the muscularis layer at E6. H is a sense control of an E10 spinal cord. Scale bar, 100 μm.
DRG at these ages and is not detectable after E12 in either the spinal cord or DRG (not shown). During the cell death period (E5.5 through E12) p75 IR is detected in spinal nerves and in the developing white matter (Fig. 1B and data not shown). Outside the nervous system, p75 IR is observed in the esophagus (Fig. 2E), as well as in the hindlimb where p75 mRNA is also present (Fig. 2G-H).

Fig. 3 shows the results of in situ hybridization studies using the \textit{trkB} probe. \textit{trkB} mRNA is not present in the spinal cord on E4 and 6 (Fig. 3A-B), but is evident throughout the gray matter, including the motor columns, on E8 (Fig. 3C-D). In contrast to p75, \textit{trkB} is expressed at high levels in a subset of DRG neurons at all ages examined (Fig. 3A-E). \textit{trkB} mRNA is also present in the nodose ganglion (Fig. 3F), the mesenchyme surrounding collecting tubules of the developing kidney (Fig. 3G), and in the developing meninges (arrowheads Fig. 3A-D).

\textit{trkB} mRNA expression in motoneurons continues after the period of naturally-occurring cell death. Fig. 4 shows that \textit{trkB} mRNA is present at E16 in a sub-population of motoneurons, commissural interneurons, cells lining the central canal, and in peripheral ganglia (not shown).

**BDNF treatment in vivo**

**Limb bud removal**

Following limb bud removal on E2, embryos were treated with 5 μg BDNF or BSA from E4.5 through E6.5 and motoneurons were counted on E7.5, at which time most untreated target-deprived neurons have already died (Fig. 5). The number of motoneurons on the side of the spinal cord ipsilateral to the limb removal is significantly lower than the number on the contralateral side, in both BDNF- \((t_{12})=16.7, P<0.01\) and BSA- \((t_{4})=56.8, P<0.01\) treated embryos and BDNF does not prevent cell death in motoneurons either ipsilateral \((t_{8})=2.1, P>0.05\) or contralateral \((t_{8})=1.5, P>0.05\) to the limb removal (Fig. 5). This, along with the temporal pattern of \textit{trkB} expression, indicates that motoneurons are insensitive to BDNF before E7.5. To test whether motoneurons are only sensitive to BDNF after E7-8 during normal development,
unoperated embryos were treated with BDNF or BSA from either E5-7 or E8-10 and cells were counted on E8 or E11, respectively. Embryos treated with BDNF from E5-7 do not have significantly higher numbers of motoneurons (t(8)=0.86; Fig. 6), whereas those treated after E8 exhibit increased motoneuron survival (t(15)=3.3, P<0.01).

**Motility and intramuscular nerve branching**

To determine if BDNF has a direct or an indirect effect on motoneuron survival, aspects of neuromuscular development other than survival were examined. Motility was measured daily for 3-5 minutes, from E6 through E9 (n = 3-5 embryos per day), both before and after 30-60 minutes treatment. BDNF had no effect on hindlimb motility either over developmental time (chronically) or over the 30-60 minutes after treatment (data not shown). Embryos examined within the first 5-10 minutes following BDNF administration also failed to show any changes in motility. These results indicate that BDNF has no effect on patterns of neuronal activity in the spinal cord or muscle (see Discussion). Furthermore, BDNF has no apparent effect on intramuscular nerve branching as shown by C2 stained nerves in BDNF- and BSA-treated sartorius muscle. There are no apparent differences in the general appearance of the nerves from either group. Counts of secondary branches of the main nerve trunks in three different hindlimb muscles (sartorius, iliouibialis and iliotibialis) are also not significantly different in BDNF- and BSA-treated embryos (data not shown).

**DISCUSSION**

The observations of p75 and trkB expression in the spinal cord described here correspond to patterns described previously by others. A number of studies (Large et al., 1989; Heuer et al., 1990a,b; Marchetti et al., 1991) have shown p75 expression in the spinal cord at early stages of chick development. We have extended these earlier studies by showing that p75 is expressed exclusively by motoneurons during early stages and only later is there expression in other areas of the gray matter. By contrast, trkB expression in the spinal cord is localized throughout the gray matter after E8 but is absent during earlier stages. Although we cannot distinguish between the full length and the truncated forms of trkB in these studies, recent studies by Escandon et al. (1994) have shown that extracts of ventral chick spinal cord at ages during the motoneuron death period contain the full length, but not the truncated forms of trkB. Therefore, the trkB hybridization shown here most likely represents full length trkB capable of signal transduction. We have not seen any hybridization signal in the E8 spinal cord in preliminary studies using a probe specific for trkB that lacks the tyrosine kinase domain (not shown).

Because trk receptors (Klein et al., 1991; Radeke and Feinstein, 1991; Holtzman et al., 1992) and p75 (DiStefano et al., 1992; Meakin and Shooter, 1992; Rabizadeh et al., 1993; Chao, 1994) can each mediate biological activities independently, it is not clear whether their expression must be temporally coordinated in neurotrophin responsive cells. Although the studies described here were not designed to test whether one or both receptors are necessary for motoneuron survival, they do show a correlation between the onset of trkB, but not p75, expression and BDNF sensitivity. While this does not rule out a role for p75 in mediating the effects of BDNF, it does indicate that trkB alone may be capable of mediating the survival effects of BDNF (Weskamp and Reinhardt, 1991). This may be true among sensory neurons as well since high levels of trkB were expressed by a sub-population of DRG neurons, presumably by cells responsive to BDNF; yet these cells express very little p75 (but see Ernfors et al., 1998).

Although the results reported here show that motoneurons are capable of responding directly to BDNF via either one or both BDNF receptors, the only way to demonstrate a direct effect of BDNF would be to examine its effects on pure cultures of motoneurons that express these receptors. Purified cultures of E15 rat motoneurons express trkB and exhibit increased survival following treatment with BDNF (Henderson et al., 1993). By contrast, cultured chick motoneurons fail to respond to BDNF (Arakawa et al., 1990; Lloyd, Newsome, Johnson, and Oppenheim, unpublished data). The pattern of trkB expression presented here may reconcile the seemingly disparate results in rat and chick. Chick motoneurons are normally prepared for culture on E5. Although p75 is expressed from E4-E10, trkB is first expressed sometime between E6 and E8. Therefore, when chick motoneurons are cultured, they are not expressing trkB and would not be expected to respond to BDNF. Furthermore, as shown here BDNF had no effect on motoneurons between E4 and E7 following limb bud removal. Since this intervention results in an almost complete loss of cells before E8, it is not surprising that BDNF was without effect. Consistent with the temporal pattern of trkB expression, we have found that motoneurons only respond to BDNF in vivo after E8 during normal development (Fig. 6) and following limb bud removal (Fig. 5 and data not shown). Oppenheim and colleagues (1992) reported that BDNF increased motoneuron survival by approx. 35%. In that study the number of motoneurons surviving on E10 following BDNF treatment was approximately 15,200, similar to the number of motoneurons normally present on E8 (approx.)
From this it seems likely that BDNF is a trophic factor for the motoneurons that die after, but not those that die prior to E8.

Temporal shifts in neurotrophic factor responsiveness have been noted among motoneurons, as well as in other systems. For example, early chick motoneurons respond to NT-3 with accelerated differentiation (Averbuch-Heller et al., 1994), but, they do not respond to NT-3 later in terms of survival (Oppenheim et al., 1993a; but see Henderson et al., 1993 in the rat). Larmet and colleagues (1992; Wright et al., 1992; Buchman and Davies, 1993) have shown that cells of the brainstem cranial nerve nuclei and peripheral ganglia have neurotrophin non-responsive periods early in development. The cells acquire neurotrophin responsiveness as they mature and begin to form contacts with targets. A similar transient period of trophic factor independence has been demonstrated for early motoneurons in vitro (Mettelting et al., 1995). Although motoneurons in vivo can not survive without trophic support before E8, the onset of BDNF responsiveness on E8 shows that motoneurons do undergo a temporal shift in sensitivity to this trophic factor.

In the chick embryo, movements of the hindlimb are directly correlated with electrical activity in the lumbar spinal cord and spinal nerves (Provine, 1971; Ripley and Provine, 1972; Landmesser and O’Donovan, 1984). Recently, Lohof and Poo (1993) and Wang et al. (1995) reported that BDNF and NT-3 promote the functional maturation of frog neuromuscular synapses in vitro. The present data on hindlimb motility in BDNF-treated embryos suggests that BDNF has no effect on neuromuscular activity. It is possible that the effects observed in vitro do not occur in vivo, or they do not occur at chicken neuromuscular junctions. Alternatively, the functional changes observed in vitro may not be reflected in increased numbers of hindlimb movements. Instead, increased neurotransmitter release or synaptic efficacy may increase the force of contraction or alter the length of time the muscle remains in the contracted state. These parameters were not assessed in our studies. Finally, BDNF may influence the function of synapses in older embryos when neuromuscular circuitry is being refined. This could explain the presence of trkB in E16 motoneurons.

Pharmacologically (e.g., curare treated) and genetically paralyzed animals have increased numbers of motoneurons, as well as increased levels of intramuscular nerve branching and synaptogenesis (Pittman and Oppenheim, 1979; Oppenheim, 1989; Dahm and Landmesser, 1988; Landmesser et al., 1988; 1990; Houenou et al., 1990, 1991). It has been suggested that the additional synapse formation augments motoneuron access to trophic factors through a mechanism involving insulin-like growth factors acting as sprouting signals (Arakawa et al., 1990; Caroni and Grandes, 1990; Oppenheim, 1989; Landmesser, 1992; Neff et al., 1993; Oppenheim et al., 1994). We examined whether BDNF might also promote motoneuron survival by increasing motoneuron branching. However, no changes in the number of sidebranches from intramuscular nerves were observed. In addition, studies of silver-cholinesterase stained muscle from BDNF-treated animals showed no obvious differences in the number or general appearance of motor endplates (not shown). Since BDNF increases motoneuron survival but not branching/synaptogenesis its mechanism of action would seem to differ from that of curare and the IGFs. However, the trkB mRNA expression observed at E16 (Fig. 4) may reflect a role for muscle-derived BDNF or NT-4, in later stages of neuromuscular development, for example, in the remodeling of connections (Funakoshi et al., 1995).

BDNF is synthesized in peripheral pre-muscle masses (Henderson et al., 1993; Hallbook et al., 1993; McKay et al., 1993), making it a potential target-derived trophic factor for motoneurons. In addition, 125I-BDNF injected into the limb bud on E8 is taken up and retrogradely transported by motoneurons (McKay et al., unpublished data), mimicking the proposed mechanism of action of target-derived trophic factors. If BDNF is a target-derived trophic molecule for motoneurons then it could be an active component of chick muscle extract (MEX), which prevents death among normal and target-deprived motoneurons (Oppenheim et al., 1988; Prevette et al., 1994). The direct test of this hypothesis, elimination of MEX bioactivity with antibodies to BDNF, has not been performed; however, two lines of evidence argue that BDNF is not the major active component of MEX. First, preparation of MEX results in less than 100-fold purification of protein, whereas when Barde and colleagues (1982) first isolated BDNF they reported that a million-fold purification was necessary to obtain micrograms of protein. It seems unlikely, therefore, that the relatively crude MEX could contain enough BDNF to have in vivo effects on motoneuron survival. Second, purified BDNF increases survival among DRG neurons, but MEX does not have the same effect (Oppenheim et al., 1988). If MEX contained BDNF then an increase in DRG neuron survival should have been observed. It should also be noted that, in addition to its presence in muscle, BDNF mRNA is detected in the chick spinal cord during the period of motoneuron death (Dechant et al., 1993; McKay et al., 1993; Escandon et al., 1994) and motoneurons that die following central deafferentation can be rescued by treatment with BDNF (Okado and Oppenheim, 1984; Oppenheim et al., 1992). Further studies are required to determine whether BDNF is a target- or an afferent-derived trophic factor for motoneurons.

The physiological relevance of BDNF as a survival factor for motoneurons has been brought into question by the results from analysis of transgenic mice. Initial studies reported that motoneuron survival was normal following targeted disruption of the BDNF gene (Ernfors et al., 1994), whereas there was a substantial loss of cells in mice lacking the trkB gene (Klein et al., 1993). Taken together these data suggested that a trkB ligand other than BDNF mediated motoneuron survival. However, deletion of the other known trk ligand, NT-4, or a double knock-out of both NT-4 and BDNF, also failed to affect motoneuron survival (Conover et al., 1995; Liu et al., 1995). In light of the apparent positive effects of trkB deletion on motoneurons (Klein et al., 1993), it was then predicted that there must be another (unknown) ligand for trkB. Surprisingly, however, a recent re-analysis of motoneuron survival in the trkB deleted mice has failed to replicate the earlier report of motoneuron loss (R. Smeyne and M. Barbadic, personal communication). Assuming that this result will be confirmed to be valid, and considering the negative results from the BDNF and NT-4 deletions, one obvious interpretation is that neither of these ligands nor their receptor are involved in the regulation of motoneuron survival. An alternative explanation is that these ligands and their receptor are, in fact, important
for the absence of BDNF, NT-4 or formal possibility that one or more of these can compensate (Oppenheim et al., 1993a,b; Hughes et al., 1993; Arakawa et al., 1990; Alderson, R. F., Alterman, A. L., Barde, Y.-A., and Lindsay, R. M. (1990).

**REFERENCES**


(Accepted 10 November 1995)