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Neurotrophin-4/5 Is a Mammalian-specific Survival Factor for Distinct Populations of Sensory Neurons

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We have studied the effect of human recombinant neurotrophin-4/5 (NT-4/5) on the survival of developing PNS neurons from embryonic mice and chickens. NT-4/5 transiently supported mouse NGF-dependent trigeminal and jugular neurons at early stages of target field innervation and mouse brain-derived neurotrophic factor (BDNF)–dependent nodose neurons during the phase of naturally occurring cell death. NT-4/5 was as potent as BDNF in supporting the survival of these neuronal populations. Surprisingly, NT-4/5 was 3 orders of magnitude less potent than BDNF as a survival factor for early chick dorsomedial trigeminal sensory neurons and did not support the survival of chick BDNF-dependent trigeminal mesencephalic or ventrolateral trigeminal sensory neurons at any of the developmental stages tested. Thus, NT-4/5 is a survival factor for certain embryonic mouse cranial sensory neurons. It is the first species-specific neurotrophin to be identified and it can discriminate at high concentrations between different BDNF-responsive chick neurons.

[Key words: neurotrophin-4/5, brain-derived neurotrophic factor, sensory neurons, survival factor, cranial neurons, cell death]

The neurotrophins comprise a family of structurally related, secreted, 13 kDa homodimeric proteins. In mammals, this family includes NGF (Levi-Montalcini and Angeletti, 1968), brain-derived neurotrophic factor (BDNF) (Barde et al., 1982; Leibrock et al., 1989), neurotrophin-3 (NT-3) (Ernfors et al., 1990; Hohn et al., 1990; Jones and Reichardt, 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990), and NT-4/5 (Berkemeier et al., 1991; Ip et al., 1992). NGF, BDNF, and NT-3 are potent survival factors for distinct sets of embryonic neurons from the CNS and PNS *in vitro* (Chun and Patterson, 1977; Johnson et al., 1986; Davies, 1987; Hatanaka et al., 1988; Hohn et al., 1990; Hyman et al., 1991; Henderson et al., 1993) and *in vivo* (Levi-Montalcini and Angeletti, 1968; Johnson et al., 1982; Kalcheim et al., 1987; Hofer and Barde, 1988; Oppenheim et al., 1992; Sendtner et al., 1992; Yan et al., 1992), mitogens for neuronal progenitors (Cattaneo and McKay, 1990; Kalcheim et al., 1992), and differentiation accelerating factors for immature neurons (Cattaneo and McKay, 1990; Sieber, 1991; Wright et al., 1992). These three neurotrophins are structurally and functionally conserved in evolution. Thus, NGF, BDNF, and NT-3 from frog and mammals share 90–95% of their primary structure (Hallbook et al., 1991) and neurons from avians, reptiles, amphibians and rodents respond equally well to the mammalian factors (Thoenen and Barde, 1980; Levi-Montalcini and Aloe, 1985; Barde et al., 1987; Hohn et al., 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990; Buchman et al., 1993). NT-4/5 (Berkemeier et al., 1991; Ip et al., 1992) is the most recently identified member of the neurotrophin family and its biological activity and species specificity have not yet been determined quantitatively. We determined the ability of recombinant NT-4/5 to promote the survival of cranial neurons from chick and mouse at different embryonic stages.

Cranial sensory neurons have been useful in studying the specificity of neurotrophins (Davies, 1987). In contrast to the functional heterogeneity of neurons in dorsal root ganglia (DRG), cranial sensory neurons are segregated into populations that deal with different kinds of sensation. Perhaps as a consequence of this functional segregation, different populations of cranial sensory neurons have distinct neurotrophin requirements when cultured during the period of naturally occurring cell death. In the chick embryo, the small-diameter cutaneous sensory neurons of the jugular ganglion and the dorsomedial part of the trigeminal ganglion (DMTG) are supported by NGF (Davies and Lindsay, 1985) but show little response to BDNF (Davies et al., 1986b) or NT-3 (Hohn et al., 1990). The large-diameter cutaneous sensory neurons of the ventrolateral part of the trigeminal ganglion (VLTG) are supported by BDNF (Davies et al., 1986b) but show little response to NGF (Davies and Lumsden, 1983) or NT-3 (Hohn et al., 1990). The proprioceptive neurons of the trigeminal mesencephalic nucleus (TMN) are supported by BDNF or NT-3 (Davies et al., 1986a; Hohn et al., 1990) but are unresponsive to NGF (Davies et al., 1987). The enteroceptive neurons of the nodose ganglion contain subpopulations of neurons that are supported by either BDNF or NT-3 (Lindsay et al., 1985; Davies et al., 1986b; Hohn et al., 1990) but are unresponsive to NGF (Lindsay and Rohrer, 1985). Cranial sympathetic and parasympathetic neurons also have distinct neurotrophin requirements. Superior cervical sympathetic ganglion (SCG) neurons are supported by NGF and NT-3 (Chun and Patterson, 1977; Greene, 1977; Rosenthal et al., 1990) and the parasympathetic neurons of the ciliary ganglion are supported by ciliary neurotrophic factor (CNTF) but not by NGF, BDNF, or NT-3 (Barbin et al., 1984; Davies et al., 1986b; Hohn et al.,

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1990). In addition, recent studies have shown that the survival of NGF-dependent cranial sensory neurons is transiently supported by BDNF and NT-3 during the early stages of target field innervation before the onset of naturally occurring neuronal death (Buchman and Davies, 1993; A. M. Buj-Bello, L. Pinon, and A. M. Davies, unpublished observations). These neurons therefore are also useful for studying the changes in requirements for neurotrophic factors during development.

Comparing NT-4/5 to other neurotrophins, we show that NT-4/5 and BDNF are survival factors for the same populations of mouse cranial neurons and that they are equipotent. In addition, we demonstrate that NT-4/5 is much less effective than BDNF as a survival factor for chick neurons and that some populations of BDNF-dependent chick neurons respond to high concentrations of NT-4/5 while others do not.

Materials and Methods

Dissection of embryonic tissues. Mouse embryos were obtained from overnight matings of CD1 mice. Pregnant females were killed by cervical dislocation and the precise stage of development of the embryos was determined by the criteria of Theiler (1972). Electrolytically sharpened tungsten needles were used to dissect trigeminal, jugular, nodose, and superior cervical sympathetic ganglia (SCG) from embryonic day 10 (E10)–E18 embryos.

Chick embryos at different stages of development were obtained by incubating white Leghorn chick eggs at 38°C in a forced-draft incubator for the required time. The trigeminal, jugular, nodose, SCG, and ciliary ganglia were dissected from chick embryos at E10 and also at E6 in the case of the trigeminal ganglion (Davies, 1989). The trigeminal ganglion was subdissected into its neural crest–derived dorsomedial pole (DMTG) and placode-derived ventrolateral pole (VLTG). The median component of the trigeminal mesencephalic nucleus (TMN) was dissected from E10 embryos (Davies, 1986).

Neuronal cultures. Embryonic mouse ganglia were incubated for 5 min at 37°C with 0.05% trypsin (Worthington) in calcium/magnesium-free Hanks balanced salt solution (HBSS). Embryonic chick ganglia were incubated for 10–15 min at 37°C with 0.1% trypsin in HBSS. After removal of the trypsin solution, the ganglia were washed twice with 10 ml of Ham's F12 medium containing 10% heat-inactivated horse serum (HIHS) and were gently triturated with a fire-polished, siliconized Pasteur pipette to give a single-cell suspension. Mouse cells and E6 chick embryo cells were directly plated at a density of 200–400 neurons per dish in 35 mm plastic tissue culture dishes (Nunc) that had been precoated with polyornithine (0.5 mg/ml, overnight) and laminin (20 μl/ml for 4 hr). Non-neuronal cells were removed from E10 chicken dissociated cell suspensions by differential sedimentation (Davies, 1986). The resulting neuronal suspensions were plated in 35 mm polyornithine/laminin-coated dishes at a density of 400–600 neurons per dish. Embryonic chick neurons were grown in F14 plus 10% HIHS. Examination of these cultures by phase contrast microscopy 48 hr after plating revealed less than 5% non-neuronal cells. Embryonic mouse neurons were grown 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 tri-iodothyronine, 60 mg/ml penicillin, and 100 mg/ml streptomycin. This medium was not conducive to the growth of non-neuronal cells, with the result that over 90% of the cells in these cultures were neurons after 48 hr of incubation.

Six to twelve hours after plating, the number of attached neurons within a 12 × 12 mm square in the center of each dish was counted; the mean of these counts was taken as the initial number of neurons in the experiment. After 48 hr of incubation, the percentage neuronal survival in the absence or presence of different neurotrophins was estimated by counting the number of remaining neurons in the same 12 × 12 mm area in each dish and expressing the results as a percentage of the initial number of attached neurons. In each experiment, triplicate cultures were set up for all conditions.

Recombinant human NT-4/5 was produced in either CHO cells or *Escherichia coli* cells. The recombinant protein was purified as described previously for NT-3 and for other neurotrophins (Rosenthal et al., 1990, 1991). Neurotrophins were added to the culture medium before plating the neurons.

Results

NT-4/5 is a survival factor for specific mouse embryo neurons

Low-density, dissociated cultures of trigeminal, jugular, nodose, SCG, and DRG neurons were established from mouse embryos between E10 and E18. These neurons were grown either in medium alone (control cultures) or in medium supplemented with NGF, BDNF, or NT-4/5. Except for nodose and jugular neurons at a very early stage of their development, virtually all neurons died in control cultures by 48 hr. Thus, the effects of neurotrophins on neuronal survival could be clearly observed at this time. Furthermore, because the serum-free medium used in these cultures prevented the growth of fibroblasts and glial cells, neuronal survival was not influenced by the release of growth factors from non-neuronal cells. For each experiment the number of neurons that initially attached to the plates was determined 6–9 hr after plating and the percentage of neurons surviving after 48 hr was calculated. As depicted in Figure 1, similar numbers of trigeminal, jugular, nodose, SCG, and DRG neurons had survived in the presence of BDNF and NT-4/5, whereas the survival response to NGF was markedly different. BDNF and NT-4/5 were both potent survival factors for E11 trigeminal and jugular neurons but were unable to support the survival of these two neuronal populations at E15. In contrast, NGF did not support the survival of E11 trigeminal and jugular neurons but was a potent survival factor for these neurons from E13 onward. The majority of nodose neurons were supported by BDNF and NT-4/5 throughout their development from E11 to E19. Less than 5% of the SCG and DRG neurons were supported by either BDNF or NT-4/5 at both E15 and E18, whereas the majority of these neurons were supported by NGF.

To determine whether BDNF and NT-4/5 act on the same subsets of trigeminal, jugular, and nodose ganglia neurons, cultures of these neurons were grown in the presence of saturating concentrations (2 ng/ml) of BDNF or NT-4/5 alone and with both neurotrophins. Cultures of E11 neurons containing individual factors or a combination of BDNF and NT-4/5 sustained the survival of a similar number of neurons for 48 hr (data not shown). The lack of an additive effect indicates that BDNF and NT-4/5 promote the survival of the same neuronal populations.

To determine whether the early survival–promoting effects of BDNF and NT-4/5 on NGF-dependent cutaneous sensory neurons are exerted over the same period of development, we compared the ability of BDNF and NT-4/5 to prevent the death of cultured trigeminal neurons at ages ranging from E10 to E14. Figure 2 shows that age-related changes in the response of trigeminal neurons to NT-4/5 and BDNF are remarkably similar. E10 and E11 neurons were the most responsive. There was a fourfold decrease in the number of responding neurons between E11 and E12, and there was a further smaller decrease in responsiveness at later ages. This indicates that BDNF and NT-4/5 are active on trigeminal neurons over the same period of development.

To investigate the relative potencies of BDNF and NT-4/5, trigeminal and nodose neurons were grown in the presence of different concentrations of BDNF or NT-4/5 and the number of surviving cells was determined 48 hr later. BDNF and NT-4/5 supported the survival of E10 and E11 trigeminal neurons to the same extent over a broad range of factor concentrations (Fig. 3*A*,*B*). Likewise, there was no significant difference in the dose responses of nodose neurons to BDNF and NT-4/5 in cultures set up at E12 (Fig. 3*C*), E15, and E18 (not shown).

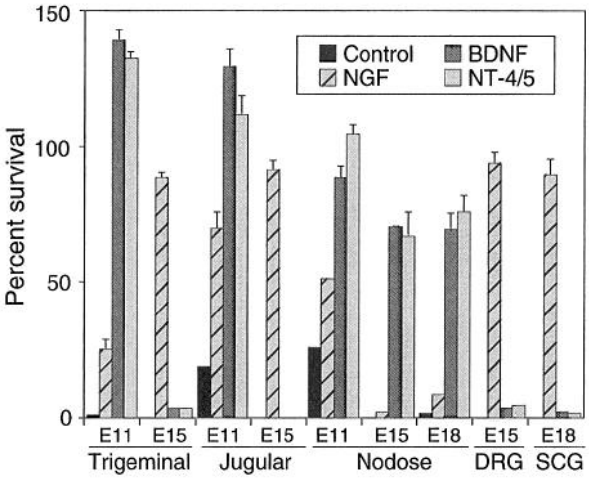

Figure 1. Survival of mouse embryo neurons in response to neurotrophins. The bar graph shows the percentage of neurons surviving after 48 hr in low-density, dissociated cultures containing 2 ng/ml of NGF, BDNF, or NT-4/5. The mean + SEM of triplicate cultures are shown.

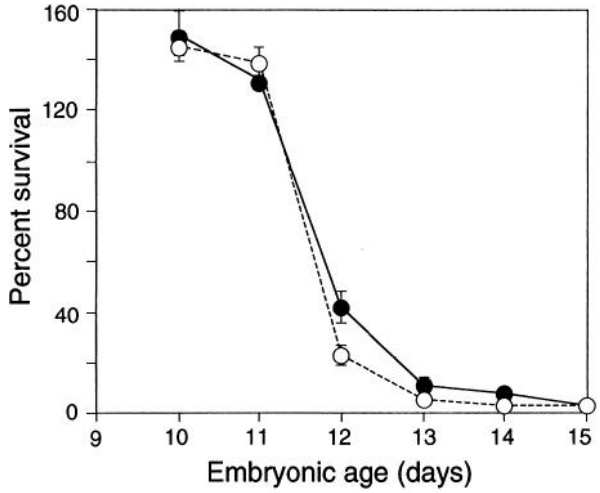

Figure 2. The survival of E10–E15 mouse trigeminal neurons in cultures containing BDNF or NT-4/5. The graph shows the number of neurons surviving after 48 hr incubation with 2 ng/ml BDNF (*open circles*) or 2 ng/ml NT-4/5 (*solid circles*) expressed as a percentage of the number of neurons identified 6–9 hr after plating. The mean ± SEM of triplicate cultures from three separate experiments at each age are shown.

Cultures of sensory ganglia from early developmental stages often contain progenitor cells that proliferate and differentiate *in vitro* (Rohrer et al., 1985: Wright et al., 1992) and it is feasible that the increase in the number of neurons in E11 cultures following neurotrophin treatment resulted from enhanced proliferation or differentiation of progenitor cells and not from the survival of post-mitotic neurons. To exclude this possibility, cohorts of neurons were identified by their bipolar morphology 6 hr after plating. BDNF or NT-4/5 was then added and the fate of the individual neurons was monitored for the next 42 hr. In these cohort experiments, 85 ± 3.8% (mean ± SEM, $n = 3$) of neurons grown with BDNF and 89.7 ± 5.2% (mean ± SEM, $n = 3$) of neurons grown with NT-4/5 survived from 6 to 48 hr in culture. In control cultures, none of the neurons identified at 6 hr survived to 48 hr. This indicates that BDNF and NT-4/5 have a direct survival-promoting effect on early trigeminal neurons. The fact that early trigeminal and jugular neurons cultured with BDNF or NT-4/5 contained more neurons at 48 hr than at 6 hr after plating (Fig. 1) suggests that proliferation or differentiation of progenitor cells occurs in these cultures. It is therefore possible that, in addition to their survival-promoting effects, BDNF and NT-4/5 act as mitogens or differentiation accelerating factors for neuronal progenitors in these cultures. Finally, although we did not undertake a quantitative study of neuronal morphology, there was no obvious difference in cell body size and in neurite length and branching between neurons surviving with BDNF and neurons surviving in parallel cultures with NT-4/5.

Effect of NT-4/5 on chick embryo neurons

The specificity and the potency of NT-4/5 were also tested on cranial sensory and autonomic neurons from E10 chick embryos. At this developmental age, cranial chick embryo neurons can be dissected as homogeneous populations and can be separated from non-neuronal cells by differential sedimentation (Davies, 1986). Furthermore, these neurons have an absolute dependence on neurotrophins for survival, and without exogenously added factors will all die by 48 hr (except for a very small percentage in SCG cultures). Surprisingly, NT-4/5 at a concentration that is above saturation for responsive embryonic mouse neurons (2 ng/ml) had no effect on any embryonic chick cranial neurons. We therefore examined the efficacy of NT-4/5 at 50 ng/ml. As shown in Figure 4, addition of NT-4/5 at this concentration had no or negligible effect on the survival of E10 BDNF-dependent TMN, DMTG, and nodose neurons, NGF-dependent DMTG, jugular, and sympathetic neurons, and CNTF-dependent parasympathetic neurons. In the same experiments, the majority of neurons were supported by 2 ng/ml of the appropriate trophic factor. Furthermore, NT-4/5 did not prevent the death of these neuronal populations even at much higher concentrations (Fig. 5). In addition, cultures containing the most effective neurotrophic factor alone or in combination with NT-4/5 (at 50 ng/ml) sustained the survival of a similar number of neurons (data not shown).

Although E10 chick neurons did not respond to NT-4/5, E6 DMTG neurons, which are BDNF-dependent at this age (Buj-Bello, Pinon, and Davies, unpublished observations), did respond to 50 ng/ml of this factor. We therefore compared the response of these neurons to the two factors (Fig. 6). Although a similar number of neurons survived in the presence of saturating concentrations of either NT-4/5 or BDNF, NT-4/5 was far less potent. For BDNF, half-maximal survival was obtained at a concentration of 7 pg/ml, while for NT-4/5, half-maximal survival was obtained at 8 ng/ml, suggesting that NT-4/5 is 3 orders of magnitude less potent than BDNF as a survival factor for E6 DMTG neurons. Thus, unlike other neurotrophins, NT-4/5 can discriminate between mouse and chick embryonic neurons. Furthermore, although all the BDNF-responsive neurons we tested in the mouse embryo are supported by NT-4/5, only subpopulations of BDNF-responsive neurons are supported by NT-4/5 in the chick embryo.

Discussion

We have quantitatively determined the ability of NT-4/5 to promote the survival of sensory and autonomic neurons of mouse and chick embryos in low-density, glia-free cultures. NT-4/5 promoted the survival of mouse E11 trigeminal and jugular

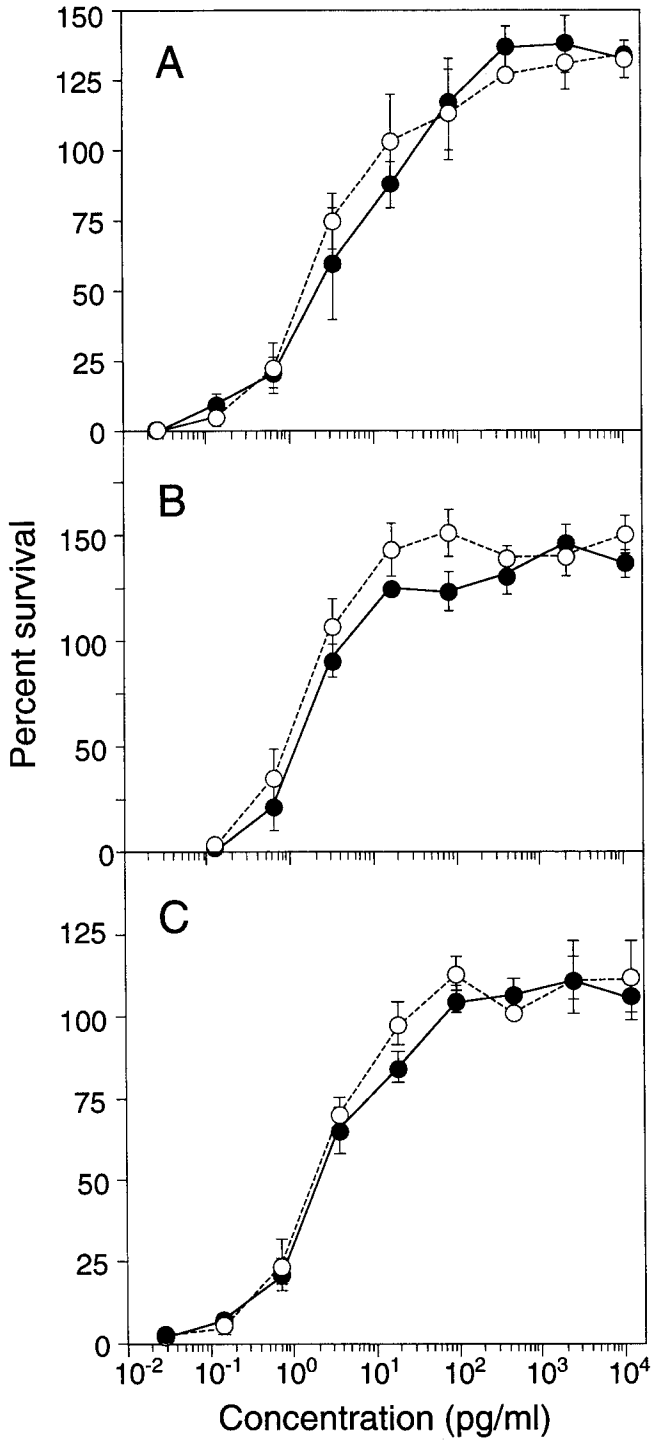

Figure 3. Dose responses of embryonic mouse trigeminal and nodose neurons to BDNF and NT-4/5: the percentage of E10 trigeminal neurons (*A*), E11 trigeminal neurons (*B*), and E12 nodose neurons (*C*) surviving after 48 hr incubation with different concentrations of BDNF (*open circles*) or NT-4/5 (*solid circles*). The number of neurons identified 6–9 hr and 48 hr after plating was used to calculate the percentage. The mean ± SEM of triplicate cultures from representative experiments are shown.

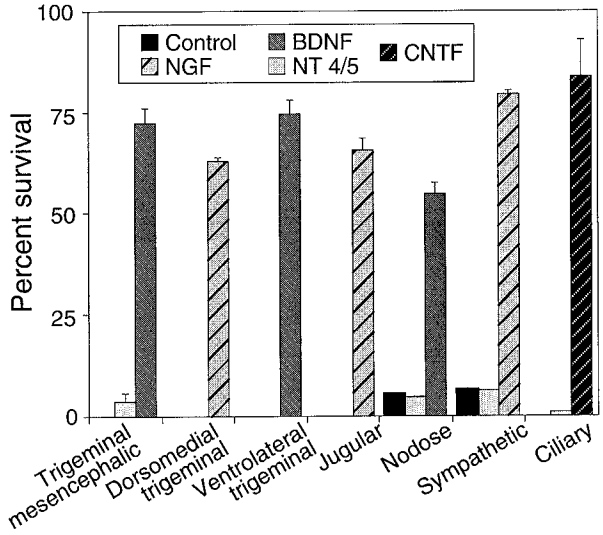

Figure 4. Survival of E10 chicken embryo neurons in response to neurotrophins. The bar graph shows the percentage of neurons surviving after 48 hr in low-density, dissociated, glia-free cultures containing no neurotrophic factors (*controls*), 50 ng/ml of NT-4/5, or 2 ng/ml of the maximally effective neurotrophic factor for each population of neurons. The mean + SEM of triplicate cultures are shown.

neurons, which are transiently dependent on BDNF at this developmental stage, and of mouse E11–E18 nodose neurons, which also depend on BDNF. NT-4/5, at the concentration tested, had negligible effect on the survival of mouse E15 and E18 DRG and SCG neurons, and E15 trigeminal and jugular neurons that are NGF-dependent at these embryonic stages. Surprisingly, although NT-4/5 and BDNF were equipotent in promoting the survival of mouse neurons, NT-4/5 was 3 orders of magnitude less potent than BDNF as a survival factor for homologous populations of chick embryo neurons. Furthermore, some BDNF-responsive chick neurons did not show any response to NT-4/5 at much higher concentrations. Thus, NT-4/5 appears to be a species-specific neurotrophin. It displays the same activities as BDNF on mammalian cranial neurons and can discriminate between different BDNF-responsive chick neurons.

One explanation for the specificity of NT-4/5 among vertebrates is that the structure of NT-4/5 was not as conserved between mammals and birds as that of other neurotrophins (Ip et al., 1992). Alternatively, it is possible that NT-4/5 fulfills requirements specific for the mammalian nervous system and is not present in birds. In addition, there may exist populations of neurons in the chick embryo that are very sensitive to human NT-4/5. Even if this were the case, we would have to conclude that there has not been selective pressure to maintain the same neuronal specificity of NT-4/5 in the avian and mammalian lineages.

Although NT-4/5 is a highly potent neurotrophin for certain populations of embryonic mouse neurons, its neuronal specificity is not unique. In our study of five different populations of neurons at stages throughout their early development, NT-4/5 displayed identical neuronal specificity and potency to BDNF. In contrast, the neuronal specificity of NGF and NT3, although partially overlapping with that of BDNF and NT-4/5 on early trigeminal and jugular neurons, was clearly different (Fig. 1; Hohn et al., 1990; Rosenthal et al., 1990; Buj-Bello, Pinon, and Davies, unpublished observations). The apparent redundancy in function of NT-4/5 observed here could be explained in several ways. First, NT-4/5 may possess unique activities on neuronal populations that have not been tested in this study. Second, although BDNF and NT-4/5 promote the survival of identical neuronal populations in culture, they may each have

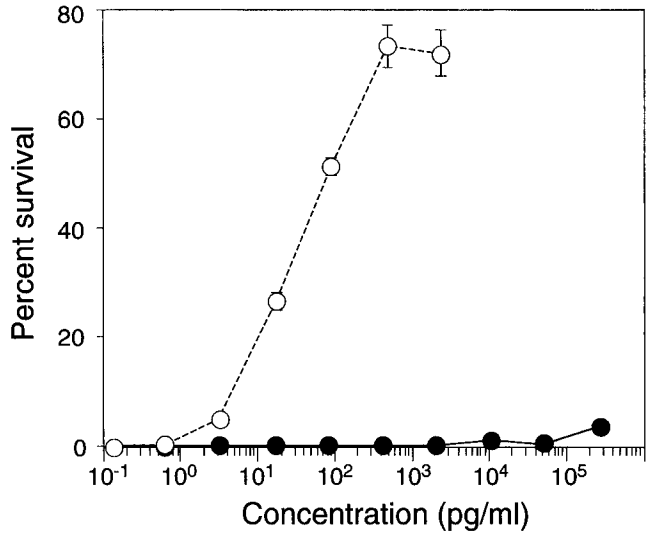

Figure 5. Dose–responses of embryonic chick TMN neurons to BDNF and NT-4/5. The number of E10 TMN neurons surviving after 48 hr incubation with different concentrations of BDNF (*open circles*) or NT-4/5 (*solid circles*) is expressed as a percentage of the number of neurons identified 6–9 hr after plating. The mean ± SEM of triplicate cultures from representative experiments are shown.

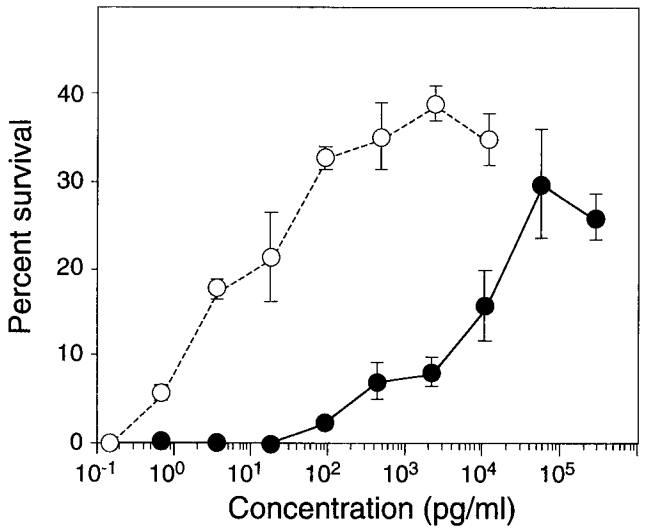

Figure 6. Dose–responses of embryonic chick DMTG neurons to BDNF and NT-4/5. The number of E6 DMTG neurons surviving after 48 hr incubation with different concentrations of BDNF (*open circles*) or NT-4/5 (*solid circles*) is expressed as a percentage of the number of neurons identified 6–9 hr after plating. The mean ± SEM of triplicate cultures from representative experiments are shown.

distinct activities *in vivo* resulting from modulation by accessory factors. Third, it is possible that the only difference between BDNF and NT-4/5 is their distinct tissue distribution. If correct, this possibility implies that amino acids that are not conserved between the two proteins are not essential for function.

The common neuronal specificity of BDNF and NT-4/5 in mouse embryos may be a consequence of the binding of these neurotrophins to the same cell surface receptor. Two classes of neurotrophin receptors have been identified: p75 and the trk family of tyrosine kinases of which three members, trkA, trkB, and trkC, have been identified (Chao, 1992; Meakin and Shooter, 1992). Considerable evidence suggests that trk receptors are directly involved in neurotrophin signal transduction and ligand discrimination. Neurotrophins promote rapid autophosphorylation of trk tyrosine kinases (Kaplan et al., 1991a,b; Klein et al., 1991a,b; Soppet et al., 1991) and elicit responses in oocytes (Nebreda et al., 1991), cell lines (Cordon et al., 1991; Glass et al., 1991; Lamballe et al., 1991; Loeb et al., 1991; Squinto et al., 1991), and embryonic neurons (T. Allsopp, M. Robinson, S. Wyatt, and A. M. Davies, unpublished observations) transfected or microinjected with *trk* cDNAs. In contrast, p75 is probably not a functional neurotrophin receptor alone (Bothwell, 1991) and binds NGF, BDNF, NT-3, and *Xenopus* NT-4 with similar affinity (Sutter et al., 1979; Rodriguez-Tébar and Barde, 1988; Hallbook et al., 1991; Rodriguez-Tébar et al., 1992). Using proliferation (Ip et al., 1992) and tyrosine phosphorylation (Berkemeier et al., 1991; L. Berkemeier and A. Rosenthal, unpublished observations) assays in 3T3 fibroblasts expressing either trkA or trkB, NT-4/5 was shown to be a potent activator of trkB and a weak activator of trkA. Like NT-4/5, BDNF also promotes rapid phosphorylation of trkB and elicits a mitogenic response in trkB-expressing fibroblasts (Glass et al., 1991; Klein et al., 1991b; Soppet et al., 1991; Squinto et al., 1991) but is inactive on trkA and trkC (Lamballe et al., 1991). Thus, it seems likely that BDNF and NT-4/5 exert their common effects on the survival embryonic mouse neurons by acting via trkB.

NT-4/5 at 50 ng/ml did not show any survival promoting effects on either E18 SCG neurons or E15 trigeminal neurons, both of which were supported by NGF. Therefore, although NT-4/5 at high concentrations can activate the trkA high-affinity NGF receptor (Hempstead et al., 1991; Kaplan et al., 1991a,b; Klein et al., 1991a; Lamballe et al., 1991; Meakin et al., 1992) in fibroblasts (Berkemeier et al., 1991; Ip et al., 1992; Berkemeier and Rosenthal, unpublished observations), this activation does not seem to have any physiological relevance for the survival of developing mouse neurons.

Previous studies of the biological activity of NT-4/5 reported that it promoted the survival of substantial numbers of embryonic chick DRG (Ip et al., 1992) and sympathetic (Berkemeier et al., 1991) neurons grown in dissociated culture. The discrepancy between these findings and our present results may have resulted from differences in the purity, processing, or concentration of NT-4/5 used. Earlier studies used partially purified conditioned media from mammalian cell lines that contained unknown concentration of NT-4/5 and could carry aggregated or truncated forms of this protein. In contrast, in the present study we used highly purified recombinant NT-4/5 at known concentrations that was produced in *E. coli.*

Although BDNF and NT-4/5 display identical specificity and potency as survival factors for mouse neurons, they differ in their ability to prevent the death of chick embryo neurons. NT-4/5, at a concentration of 50 ng/ml, promoted the survival of E6 DMTG neurons but was an ineffective survival factor for E10 TMN neurons. In contrast, BDNF displayed similar potency in promoting the survival of both of these neuronal populations and was more potent than NT-4/5. Thus, NT-4/5 and BDNF differ in both specificity and potency as survival factors for chick embryo neurons. Although E6 DMTG neurons respond to NGF (Buj-Bello, Pino, and Davies, unpublished observations) whereas E10 TMN neurons are unresponsive to NGF (Davies et al., 1987), it is unlikely that NT-4/5 exerts its effects on E6 DMTG neurons via a regular *trkA* receptor because it

does not support the survival of other NGF-dependent neurons. Alternatively, it is possible that E6 DMTG and E10 TMN neurons express different forms of the *trkA* or *trkB* receptors or of p75-like accessory molecules. Multiple *trkB* transcripts and at least three distinct *trkB* receptors that differ in their cytoplasmic domains have already been identified (Klein et al., 1989, 1990; Middlemas et al., 1991). The ability of human NT-4/5 to discriminate between different *trkB*-responsive chick neurons suggests another level of complexity in the interactions between neurotrophins and their receptors.

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