Sensory ganglia require neurotrophin-3 early in development

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SUMMARY

The role played by neurotrophin-3 during the development of quail sensory ganglia was investigated using a monoclonal antibody that specifically blocks the biological activity of this neurotrophin. Neutralisation of neurotrophin-3 was initiated during completion of gangliogenesis. Neuronal cell counts indicate that about 30% of the neurons normally present in either the placode-derived ganglion nodosum or in a leg-innervating, neural crest-derived dorsal root ganglion are eliminated by the antibody treatment. In both ganglia, this reduction is seen early in development, and the results obtained with the ganglion nodosum indicate that neurotrophin-3 plays an essential role already during gangliogenesis. Neuronal numbers are also compared with those obtained after treatment with a monoclonal antibody to nerve growth factor, used either alone or in combination with the neurotrophin-3 antibody.

Key words: neurotrophins, sensory ganglia, cell death, neurogenesis

INTRODUCTION

For questions that relate to mechanisms of nervous system development in vertebrates, the peripheral sensory ganglia are convenient and interesting structures to study (for review, see Perl, 1992). These ganglia are composed of relatively small numbers of neurons that can be readily counted, and of a reasonably diverse repertoire of neuronal phenotypes that can be categorised into subsets according to functional, as well as anatomical and biochemical criteria. How neuronal numbers are controlled and how the phenotypic diversity arises during development are amongst the problems that can be approached. While local cues are likely to play important roles in the generation of the various types of sensory neurons, their molecular nature is still largely unknown (for review, see Le Douarin et al., 1992). However, it is becoming apparent that members of the nerve growth factor family - the neurotrophins - might be interesting candidates to study in this context. For example, the neurotrophins all prevent the death of primary sensory neurons in cell culture experiments (for review, see Davies, 1992). Typically, neurotrophins prevent cell death in vitro when neurons are isolated during the time when normally occurring neuronal death is observed in vivo in the corresponding ganglia. Correlations have also begun to be established between the neurons rescued in vitro by one particular neurotrophin and their pattern of projections in vivo. For example, antibodies to the carbohydrate epitope SSEA-1 have been used in vitro to correlate the neurons rescued by BDNF with labelling patterns in the dorsal root ganglia (DRG) and their projections in the spinal cord (Scott, 1993). Also, retrograde labelling of muscle afferents has revealed that NT-3 preferentially rescues this sub-population of primary sensory neurons in vitro (Hory-Lee et al., 1993). Finally, BDNF has been shown, in vitro, to favour the differentiation of pluripotent neural crest cells towards the sensory lineage, suggesting the possibility that some neurotrophins might have early actions, not related to neuronal survival (Sieber-Blum, 1991).

Very few results are available regarding the roles of neurotrophins during normal development, with the exception of NGF. Thus, NGF antibodies, when administered to quail embryos, decreased neuronal numbers in neural crest-derived DRGs, but not in the nodose ganglia (NG; Rohrer et al., 1988), in line with similar observations previously made in rodents (Pearson et al., 1983). Results of this sort have been important in indicating that NGF plays an essential role in regulating cell numbers in neural crest-derived peripheral ganglia, following early studies on the role of NGF in the developing sympathetic system (Cohen, 1960; Levi-Montalcini and Booker, 1960).

The role played, during the development of peripheral ganglia, by neurotrophins other than NGF is much less clear. While it has been shown that BDNF, when administered to developing quails and chicks, prevents neuronal death in NG and DRG (Hofer and Barde, 1988; Oppenheim et al., 1992), it remains to be demonstrated that BDNF deprivation would lead to the opposite result. Such a role appears possible, since the targeted elimination of one of the tyrosine kinase-coding exon of trkB, a functional receptor for BDNF and NT-4/5, which also binds NT-3 (for review see Meakin and Shooter, 1992), leads to marked neuronal losses in the mouse trigeminal and dorsal root ganglia when cell counts are performed in newborn mice that are homozygous for the deletion (Klein et al., 1993). Effects of NT-3 in the intact developing animal have not yet been reported. Results of in vivo experiments indicating an essential role for the new neurotrophins are also important in view of the many structural and functional similarities between the neurotrophins. All 4 (or 5) neurotrophins have been shown to act on peripheral sensory neurons, and at least in vitro, redundancy has clearly been documented. For example, essen-
Female Balb/c mice were immunised (and boosted) intraperitoneally with 15-20 µg of NT-3 in phosphate-buffered saline emulsified with Freund’s adjuvant (Gibco). For fusion, one of the immunised mice was selected after the third boost. When used at a dilution of 1:3,000 in survival assays with nodose neurons, its serum completely blocked the activity of NT-3 (1 ng/ml), but not that of BDNF (1 ng/ml), even at a dilution of 1:200. 3 days before fusion, the mouse received three intravenous injections of NT-3 in phosphate-buffered saline (about 20 µg each). The fusion of the spleen cells with a non-secreting mouse myeloma cell line (X63.Ag 8.635) was accomplished using 50% (w/v) polyethylene glycol 4000. Positive clones were selected by an immunoassay using an anti-mouse IgG antibody and cloned using a cell sorter. Plates were coated with 10 ng NGF or NT-3 and supernatants were selected for high signals against NT-3, and background signals against NGF. The anti-NT-3 antibody designated no. 12 (mAb no. 12) used in all experiments belongs to the IgG1 subclass, as determined using class-specific antibodies.

**Antibody treatment**

The in vivo antibody treatment involves the in ovo growth of hybridoma cells following the procedure described by Rohrer et al., 1988. The shell of fertilised quail eggs, incubated at 37°C in 70% relative humidity, was opened during the third day of incubation, at stage 14 or 15. After removing the shell membrane, 1-1.5×10⁶ cells of hybridoma clone secreting mAb no. 12 or the anti-NGF monoclonal antibody 27/21 (mAb no. 27/21) (Korsching and Thoenen, 1987), or both, were suspended in 50 µl phosphate-buffered saline (pH 7.3) and were placed onto the chorioallantois membrane.

**Histological procedures**

Embryos were staged according to Zacchei, 1961 and the ciliary ganglion, the NG and DRG no. 25 were dissected, fixed in 4% paraformaldehyde, embedded in paraffin and sectioned at 6 µm. Sections were stained with cresyl violet. The neurons were counted in every other section at a magnification of ×400. Neurons were identified by their size, staining intensity and the structures counted were cytoplasmic profiles meeting these criteria and containing in addition a nucleus and at least one nucleolus, usually appearing as a very condensed, dark structure (see also Hofer and Barde, 1988). About 16% of the largest, and 12.5% of the smallest DRG neurons were found to contain more than one nucleolus.

**Measurements of mouse antibody levels in quail tissues**

The levels of antibody in tissues (brain was used in most cases) were determined using a 2-site enzyme immunoassay (ELISA). F(ab)2 fragments of affinity purified anti-mouse IgG antibody developed in sheep (Sigma) or horse antibodies (as controls for the determination of non-specific binding) were bound to 96-well plates. The supernatants of the tissue samples (homogenised in 0.1 M Tris-HCl, pH 7.0 containing 0.3 M NaCl and 0.2% bovine serum albumin) were diluted in a solution containing 10 mM MgCl₂ and 0.2% Triton X-100. The detection antibody was a polyclonal goat anti-mouse-IgG-β-galactosidase conjugate (Southern Biotechnology Associates, Inc.) used at a dilution of 1:8,000, incubated for about 30 minutes at room temperature with 200 µM of the β-galactosidase substrate 4-methylumbel liferyl-β-D-galactoside. A standard curve was constructed covering the range 0.2-16 ng IgG per ml using known quantities of purified mAb no. 27/21. Tissues from non-treated quails did not give a measurable signal in this immunoassay.

**Neuronal cultures**

NGs and DRGs were isolated form chick embryos at E7 and E9 respectively. The ganglia were dissociated with trypsin, pre-plated to remove most of the non-neuronal cells, and plated onto 48-well plates at a density of about 2,000 cells per well onto a polycornithine substrate pre-coated with medium conditioned by RN22 Schwannoma cells (see Rodriguez-Tébar et al., 1990). The culture medium was F-14 to which 10% heat-inactivated horse serum was added. Neutrophins were used at a concentration of 1 ng/ml and the monoclonal antibodies at 1 µg/ml (no. 27/21, purified by Protein-A Sepharose affinity chromatography) or 40 µg/ml (no. 12, purified by ion-exchange chromatography). The number of surviving neurons was determined after 20-24 hours in culture and is expressed as the percentage of neurons initially plated, as determined within the first 2 hours following plating.

**MATERIALS AND METHODS**

**Neurotrophins**

NT-3 antibody-secreting cells in quail embryos. We also compared the effects with those observed using a monoclonal antibody to NGF either alone or combined with the NT-3 antibody.
RESULTS

Specificity of the monoclonal anti-NT-3 antibody no. 12 (mAb no. 12)
The specificity of mAb no. 12 was tested in a survival assay using sensory neurons isolated from chick NGs and DRGs. BDNF, NT-3 and NT-4 all prevent the death of a proportion of the NG neurons. mAb no. 12 was found to completely inhibit the effects of NT-3 on these neurons, but not those of BDNF or NT-4 (Fig. 1). The monoclonal anti-NGF antibody no. 27/21 (mAb no. 27/21) did not inhibit the effects of any of the 3 factors. Using DRG neurons, mAb no. 12 was found not to interfere with the effects of NGF, whereas as expected, mAb no. 27/21 completely prevented the NGF-mediated survival effects (Fig. 2). These results thus indicate that the 2 monoclonal antibodies used in this study not only block the biological activities of NT-3 and NGF, but also block these activities specifically.

Fig. 1. Survival of nodose neurons after 1 day in culture. Neurotrophins (1 ng/ml) were added either alone or together with a monoclonal antibody against NT-3 (no. 12) or against NGF (no. 27/21). Note that the antibody no. 12, but not the antibody no. 27/21, blocks the effects of NT-3. Neither antibody blocks the effects of BDNF or Xenopus NT-4. Results are means of 3 different wells ± s.d. and are representative of 5 different experiments.

Fig. 2. Survival of dorsal root ganglion neurons after 1 day in culture. NGF was used at 1 ng/ml. Note that the antibody against NT-3 (no. 12) does not affect the neuronal survival observed with NGF. Results are means of 3 different wells ± s.d. and are from 3 different experiments.

In vivo effects of mAb no. 12 applied alone or combined with mAb no. 27/21
In order to assess the effects of NT-3 deprivation during development, hybridoma cells secreting mAb no. 12 were applied onto the chorioallantois membrane of quails during the third day of incubation, when neuronal numbers are still increasing in both the NG and the DRG (Hofer and Barde, 1988). We wanted first to determine if the NT-3 antibody would lead to a reduction in cell numbers in sensory ganglia beyond that normally seen when the period of normally occurring cell death is completed. Such a result would be predicted on the basis of numerous previous in vitro experiments, indicating that NT-3 can prevent neuronal death in culture (see for example Hohn et al., 1990; Hory-Lee et al., 1993; see also Fig. 1). In the several dozen quails treated with mAb no. 12 and examined at E11, no obvious and reproducible differences were seen between these quails and animals treated with mAb no. 27/21. In particular, neither the developmental stages (according to external morphological criteria), nor the weight revealed consistent differences when mAb no. 12-treated embryos were compared with mAb no. 27/21 embryos. The ganglia examined in this study were not obviously smaller, but when placed side by side, NGs or DRGs from mAb no. 12 antibody-treated quails were slightly smaller than ganglia from control animals. Neuronal numbers were determined at E11 in sections of DRG no. 25 and the NG (Table 1). About 28% and 36% of the neurons were missing in the NG and the DRG respectively in embryos treated with mAb no. 12 compared with untreated embryos. No reduction was observed in the ciliary ganglion. The amount of antibody was also determined in quails treated with the mAb no. 12-secreting hybridoma cells using an immunoassay allowing mouse immunoglobulins to be quantified. The values obtained were high, in excess of 30 µg/g wet weight in several animals at E11. Variations in antibody concentrations were observed between embryos, but no correlation was found between antibody titres and neuronal numbers (2783 and 2801 neurons in the NG of 2 quails with antibody titres of 24 and 70 µg/g wet weight respectively). We next determined the effects on neuronal numbers of both mAb no. 27/21 and mAb no. 12 combined. We found that while, as expected, the number of NG neurons was not reduced beyond

Table 1. Neuronal numbers in E11 quail ganglia after antibody treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NG</th>
<th>DRG</th>
<th>Ciliary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3803±48 (n=6) (100%)</td>
<td>4597±109 (n=7) (100%)</td>
<td>2807±16 (n=4)</td>
</tr>
<tr>
<td>mAb no. 12</td>
<td>2753±85 (n=5) (72.3%)</td>
<td>2956±221 (n=7) (64.3%)</td>
<td>2890±133 (n=4)</td>
</tr>
<tr>
<td>mAb no. 27/21</td>
<td>3757±126 (n=5) (98.7%)</td>
<td>3215±333 (n=5) (69.9%)</td>
<td></td>
</tr>
<tr>
<td>mAb no. 12 + mAb no. 27/21</td>
<td>2931±254 (n=6) (75%)</td>
<td>2443±323 (n=4) (53%)</td>
<td></td>
</tr>
</tbody>
</table>

Means are ± standard deviations, ganglia are from different animals. *Different from control or mAb no. 27/21 (anti-NGF) alone at P<0.001 (unpaired t-test). †Different from control at P<0.001. ‡Different from either mAb no. 27/21 or mAb no. 12 (anti-NT-3) at P<0.1, from control at P<0.001.
the value obtained with mAb no. 12 alone, the number of DRG neurons was decreased beyond what is observed when either antibody is used singly (Table 1): compared with control embryos, 47% fewer DRG neurons were found (mAb no. 12 alone: 35.7%, mAb no. 27/21 alone: 30.1%). The reduction observed with both antibodies combined is, however, not as large as would be expected if the NGF and NT-3-dependent populations were entirely distinct.

Early effects of mAb no. 12 compared with mAb no. 27/21

Having established that the anti-NT-3 antibody induces a reduction in cell numbers beyond the decrease observed in either ganglia after the period of normally occurring neuronal death, we were interested to see if this decrease could be observed when maximal neuronal numbers are reached in these ganglia, at E5 for the NG and at E6 for DRG no. 25. The results of this experiment clearly indicate that such is the case (Table 2). In contrast, using the anti-NGF antibody, only a very small reduction is seen in the DRG at E6 (Table 2). These results indicate that NT-3 is necessary very early in the formation of the NG, and that in the DRG, where the effects of both antibodies can be compared, many more neurons require NT-3 early than NGF.

DISCUSSION

Biological effects of monoclonal antibodies in vivo

The main aim of this study was to determine whether NT-3 is required for normal development of peripheral sensory ganglia. Hybridoma cells secreting a monoclonal antibody specifically blocking the biological activity of NT-3 were grown in ovo and applied during completion of gangliogenesis. The simplest conclusion that can be drawn is that NT-3 is indeed required for the normal development of peripheral sensory ganglia, fewer neurons than normal being found in either the placode-derived NG or in the neural crest-derived DRG. We feel that it is unlikely that the effects we observed are due to reasons other than the neutralisation of endogenous NT-3 by the antibody. Firstly, the number of ciliary neurons (known from in vitro experiments not to be supported by NT-3) remains unchanged when antibody-treated quails are compared with untreated embryos. Secondly, the NGF antibody (of the same immunoglobulin class) offers a relevant point of comparison, since the effects obtained differ from those observed with the NT-3-antibody. While in most animals, the titres reached with the NGF antibody (mean: 12 µg/g wet weight in 9 embryos) were somewhat lower than those reached with the NT-3-antibody, in about 10% of the animals the titres were virtually identical or higher in the case of the NGF antibody. Neither with the NT-3 nor with the NGF antibody did we find any correlations between antibody titres and neuronal numbers in individual embryos. It thus appears that the antibody concentrations reached are beyond those necessary to neutralize endogenous NT-3 or NGF.

In this study, we used a monoclonal antibody raised against mouse NT-3, but quail NT-3 is unlikely to be very different from the rodent antigen. Indeed, the sequence of NT-3 corresponding to the biologically active molecule is well conserved, the amino acid sequence of all mammals analysed so far being 100% identical. In chick NT-3, there is only one conservative amino acid exchange compared with mammals (Hallböök et al., 1993). While quail NGF is likely to differ in sequence from mouse NGF (19 amino acid replacements in chick compared with mouse NGF), cross-reactivity of the anti-mouse NGF antibody used in this study with the avian antigen has been demonstrated in biological assays (Rohrer et al., 1988), as well as in immunoprecipitation and immunoassay studies (Ibáñez et al., 1991).

How can the availability of NT-3 influence neuronal numbers during development? The most obvious explanation for this result would seem to be that in analogy with the model elaborated for NGF, sub-populations of sensory neurons absolutely need NT-3 if they are to survive past the time when the process of normally occurring neuronal death starts to operate, eliminating in control animals 35% (NG) and 43% (DRG no. 25) of the neurons between E5 and E11 (Hofer and Barde, 1988 and Tables 1 and 2). This interpretation is also suggested by the results of in vitro experiments demonstrating that NT-3 does prevent the death of sub-populations of neurons isolated from the corresponding ganglia during the time of normally occurring cell death (see for example Fig. 1). So far however, there are no in vivo data indicating that the administration of NT-3 prevents normally occurring neuronal death (unlike with NGF and BDNF). In fact, a close analysis of our data suggests that NT-3 might also regulate neuronal numbers by mechanisms operating before those controlling cell death by the availability of target-derived survival molecules. This is especially striking when the data obtained with the NG are analysed.

Reduction in neuronal numbers in the nodose ganglion

In the NGs of embryos treated with the anti-NT-3 antibody, 34% of the neurons are already missing at E5 (stage 20, Table 2). Cell counts at an even earlier time point, E4.5 (stage 19) similarly revealed a 33% reduction in neuronal numbers. How can this finding be explained? Previous in vitro experiments with chick neurons isolated from the NG at stage 19/20 (corresponding to stage 15 in the quail, when we start the antibody treatment) have indicated that these neurons can survive in culture for extended periods of time in the absence of BDNF or NT-3 (Vogel and Davies, 1991). In fact, the addition of these neurotrophins only begins to have measurable effects on neuronal survival after 72 hours in vitro (Vogel and Davies, 1991). The neurotrophin-independent survival of NG neurons, which extends over a particularly long period of time compared...
with other cranial ganglia, has been correlated with the comparably long distances that the axons have to cover to reach their targets (Vogel and Davies, 1991). However, during this developmental period, our results indicate that NT-3 is needed in vivo if the full complement of neurons is to be reached in the NG. Thus, NT-3 appears to be essential in vivo for a function other than the regulation of the survival of cells identifiable as neurons at the time of culture. NT-3 might regulate neuronal numbers either by stimulating the division of neuronal precursors (which are abundant at these early stages in the NG, but do not survive well in vitro as dividing cells, see Rohrer and Thoenen, 1987; Rohrer, 1990), or by stimulating, in vivo, the differentiation of such precursors into sensory neurons that would either die in the absence of NT-3 or differentiate into cells other than neurons. Recent in vitro results suggest that such possibilities are likely. Firstly, it has already been observed using E4.5 (stage 25/26) chick DRG that NT-3, but not NGF or BDNF, induces the generation of some new neuronal profiles in serum-free cultures and that this result can be explained by promotion of cell division or of cell differentiation, but not of neuronal survival (Wright et al., 1992). Similarly, NT-3 (but not BDNF) has recently been shown to promote the differentiation and maturation of retinal precursor cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimulate the division of neural crest cells in vitro (particularly when cells (de la Rosa et al., 1994). Secondly, NT-3 can stimul

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Effects of antibodies to NT-3 and NGF on cell numbers in the DRG

It has long been known that in the avian DRG, even though all neurons derive from the neural crest, they do not differentiate as a homogenous group of cells. In particular, studies in which not only the number, but also the location of degenerating neurons have been assessed, have revealed differences between the large lateroventral neurons, and the small mediadorsal one, most neurons degenerating in the former population about 3 days earlier than in the latter (Hamburger et al., 1981; McMillan Carr and Simpson, 1978a). It is also known that injections of NGF, while completely preventing normally occurring neuronal death in the dorsomedial neurons, only partially reduces it in the lateroventral population (Hamburger et al., 1981), though this population has long been known to be target-dependent (Hamburger and Levi-Montalcini, 1949; McMillan Carr and Simpson, 1978b). Retrograde transport studies using the tracer horseradish peroxidase (injected either in the skin or in the muscle) have indicated that the lateroventral neurons are the first to differentiate and can be labelled as early as E5 (Honig, 1982). In view of these results, it is likely that part of the 34% reduction we see in the E6 DRG after anti-NT-3 treatment results from the elimination of these early differentiating and projecting neurons, presumably including the sub-population that has been identified as muscle afferents in the chick DRG at E8 and that is specifically supported by NT-3 in vitro (Hory-Lee et al., 1993). However, the comparatively large losses we observe probably also result from neurons that could not be generated for the same reasons as those discussed for the NG. The trkC mRNA expression data in the quail DRG give support to these interpretations. Many cells were found to express trkC at E3, and there is a rapid restriction of the expression pattern to some of the large neurons in the lateroventral population over the following days (Zhang et al., 1993). The NGF antibody produces hardly any significant reduction in cell numbers at this stage which is in line with the suggestion that the early differentiating neuronal population is largely NGF-independent. Subsequently, the NGF antibody does indeed eliminate significant numbers of DRG neurons (about 30%), as already documented in a previous study (Rohrer et al., 1988).

The analysis of the results obtained at E11 with both antibodies combined suggests that while the effects on cell numbers are larger (47%) than those observed with either antibody used alone (36% for anti-NT-3, 30% for anti-NGF), the effects are not fully additive, since a decrease by about 66% would have been expected for 2 fully distinct NT-3 and NGF-sensitive populations (see Table 2). A likely explanation for this observation is that a significant proportion of the neurons depends on NT-3 to be generated in the first place (like some NG neurons), and only subsequently on NGF for their survival, when these neurons are fully differentiated and their axon terminals seek NGF in the peripheral targets. Such a switch in neurotrophin dependency has recently been demonstrated with cultured neurons isolated from rodent peripheral ganglia. Within 3 days (E10-E13) in the mouse trigeminal ganglion, this neuronal population shifts from an absolute dependency on NT-3 or BDNF, for survival in culture, to dependency on NGF (Buchman and Davies, 1993). Similarly, in the developing rat sympathetic ganglion, there is a shift from NT-3 to NGF between E14.5 and P0 for survival in vitro (Birren et al., 1993).

Conclusion

Our results indicate that a reduction in NT-3, but not in NGF levels, when imposed during completion of gangliogenesis,
rapidly and markedly affects neuronal numbers in sensory ganglia. The data obtained with the NG, together with previous in vitro results with neurons isolated from this ganglion, indicate that NT-3 is essential early in development, before the target-derived, neurotrophin-mediated regulation of neuronal survival. Later during development, NT-3 might also act as a necessary target-derived neurotrophin supporting the survival of small subsets of primary sensory neurons, including muscle afferents (Hory-Lee et al., 1993). These data suggest that the neurotrophins might have in vivo roles during the formation of peripheral ganglia that cannot be anticipated from what has been learned so far with NGF.

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