

Specific High-Affinity Receptors for Neurotrophin-3 on Sympathetic Neurons

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When used at concentrations allowing interactions only with its high-affinity receptors, neurotrophin-3 (NT-3) promotes the survival of sensory neurons isolated from embryonic day 8 (E8) chicks, but not the survival of E11 sympathetic neurons. These sympathetic neurons (which can be rescued by the addition of NGF) display high-affinity receptors for NT-3 (K_d of 1.6×10^{-11} M) that cannot be distinguished from the high-affinity NT-3 receptors on sensory neurons using equilibrium binding or kinetic criteria. This represents the first example of embryonic neurons that cannot be rescued by the *in vitro* addition of a neurotrophin in spite of the presence of corresponding neurotrophin high-affinity receptors. At elevated concentrations, beyond the saturation of its high-affinity receptors, NT-3 supports the survival of some E11 sympathetic neurons, an effect that might be mediated by the high-affinity NGF receptor. Using E7 sympathetic neurons, about 40% of the cells initially plated can be rescued *in vitro* by the addition of low concentrations of NT-3 (but not of NGF) and produce profuse neurites. This indicates that NT-3 may play a role in the early development of some sympathetic neurons.

[Key words: neurotrophins, NGF, neurotrophin-3, binding studies, sympathetic neurons, neuronal survival]

Many populations of vertebrate neurons are influenced by regulatory signals located in the tissues that they innervate (for review, see Purves, 1988). This can be most dramatically illustrated during development: ablation of the target tissue leads to the death of the neurons that would have innervated it (for a review, see Oppenheim, 1991). Currently, the most plausible mechanistic explanation that can be given for this observation is that the target cells secrete specific proteins able to stop a death program operating in the embryonic neurons at the time of target innervation (for recent reviews, see Barde, 1989; Ellis et al., 1991; Raff, 1992). Pioneering work on the neurotrophic protein NGF has been crucial in the elucidation of many aspects of this concept (for reviews, see Levi-Montalcini and Angeletti, 1968; Thoenen et al., 1987). More recently, a second neurotrophic protein, brain-derived neurotrophic factor (BDNF), has

been shown *in vivo* to prevent the death of neurons during development in NGF-independent neuronal populations (Hofer and Barde, 1988). The comparison of the primary structure of BDNF with that of NGF revealed a substantial degree of similarity, a finding that led to the suggestion that the structural relatedness between NGF and BDNF might be used for the characterization of additional members of this gene family (Leibrock et al., 1989). The list of NGF homologs did indeed grow by the subsequent addition of neurotrophin-3 (NT-3; Ernfors et al., 1990; Hohn et al., 1990; Jones and Reichardt, 1990; Kaisho et al., 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990) and neurotrophin-4 (NT-4; Hallböök et al., 1991). Until now little is known about the newest neurotrophic factors. Recombinant NT-3 has been shown to promote the *in vitro* survival of some primary sensory neurons (Hohn et al., 1990; Kaisho et al., 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990). NT-3 receptors have been identified in binding studies using dorsal root ganglia (DRG) neurons (Rodríguez-Tébar et al., 1992). Two classes of NT-3 receptors could be distinguished: high-affinity receptors (K_d of 0.9×10^{-11} M) that have the property to readily discriminate between NGF, BDNF, and NT-3, and low-affinity receptors (K_d of 0.4×10^{-9} M), corresponding most probably to the well-characterized low-affinity NGF, or neurotrophin, receptor (LANR).¹ These results were in many ways reminiscent of those obtained in previous binding studies with BDNF and NGF using DRG neurons (Sutter et al., 1979; Rodríguez-Tébar and Barde, 1988).

So far, the presence of high-affinity NGF or BDNF receptors, as opposed to that of low-affinity receptors, has been a reliable predictor of a neuronal survival response *in vivo* and *in vitro*. Reasoning by analogy, it is tempting to use the presence of high-affinity NT-3 binding sites on neurons as an indication for a survival function of NT-3 on the neurons displaying such receptors. In our previous binding study with NT-3, however, a surprisingly large number of high-affinity NT-3 binding sites were found on DRG neurons. While supporting the survival of fewer embryonic day 8 (E8) DRG neurons than BDNF or NGF, NT-3 was found to have as many as 5000 high-affinity binding sites on these neurons (Rodríguez-Tébar et al., 1992), about 10 times more than the number of high-affinity NGF or BDNF receptors (Sutter et al., 1979; Rodríguez-Tébar and Barde, 1988). This finding led to the speculation that high-affinity NT-3 re-

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¹Because recent results indicate that NT-3 and BDNF, like NGF, form stable dimers (Radziejewski et al., 1992), we now express the molar concentrations of these neurotrophins, the K_d values, and numbers of receptors based on the molecular weight of the dimers (as opposed to the monomers in our previous studies with BDNF and NT-3).

ceptors might exist that are not mediating a neuronal survival response. In the present study, we tested this possibility using sympathetic neurons. We found numerous high-affinity NT-3 receptors on E11 neurons, but no survival response using concentrations of NT-3 high enough to saturate these receptors.

Materials and Methods

Materials. Recombinant NT-3 was produced and purified as described (Götz et al., 1992). 2.5S NGF was prepared from adult male mouse submandibular glands (Suda et al., 1978). The anti-NGF monoclonal antibody 27/21 was purified using protein A-Sepharose and was added to the cultures together with NGF and/or NT-3 as described in Results.

Radiolabeling. NGF and NT-3 were labeled using the lactoperoxidase methods as described previously (Rohrer and Barde, 1982; Rodríguez-Tébar et al., 1992). For NT-3, about 2.5 μ g of the protein was incubated for 45 min at 0°C in a total volume of 30 μ l of 0.1 M sodium phosphate (NaPi) buffer (pH 7.2) containing 0.2 μ g of lactoperoxidase (Sigma; 80 U/mg), 400 μ Ci of 125 I-Na (2000 Ci/mmol; New England Nuclear), and 170 μ M H_2O_2 . At the end of the incubation period, 85–95% of the total radioactivity could be precipitated with trichloroacetic acid. Subsequently, the reaction was stopped by dilution with the NaPi buffer to 250 μ l. After 12 hr at 4°C, 3 mg of bovine serum albumin was added to the reaction mixture. The mean specific activity of 125 I-NT-3 was found to be 130–160 cpm/pg protein, corresponding approximately to one radio-atom per monomer of NT-3. 125 I-NT-3 was used within 7 d after the reaction. Radiolabeled NGF was used within 3 d after preparation. The radiolabeled neurotrophins were found to have the same specific biological activity as the native proteins in a bioassay using E8 chick embryonic dorsal root ganglion neurons.

Primary culture. For neuronal cell cultures, lumbosacral sympathetic chains from E7 and E11 chick embryos were isolated. E7 sympathetic cells were cultured on four-well dishes (Greiner) at a density of 1500 cells per well on a polyornithine-laminin substrate in F-14 medium containing 10% heat-inactivated horse serum plus 5% fetal calf serum (Ernsberger et al., 1989). Phase-bright cells were counted at the time points indicated in Results. E11 sympathetic neurons were cultured as described (Lindsay et al., 1985) and neurite-bearing cells were counted after 72 hr.

Cell preparation for binding studies. For binding experiments, 50–100 lumbosacral sympathetic chains (E7 or E11) were isolated, the ganglia dissociated, and the neurons obtained as described (Rodríguez-Tébar and Barde, 1988). Briefly, the chains were collected in ice-cold F-14 medium containing 10% heat-inactivated horse serum. The sedimented ganglia were centrifuged, resuspended in 5 ml of Ca^{2+}/Mg^{2+} -free, prewarmed Gey's buffer (Gey and Gey, 1936), and incubated for 10 min at 37°C with trypsin (Worthington) at a final concentration of 0.1 mg/ml. Trypsinization was terminated by the addition of 10% horse serum. The tube was placed on ice and the ganglia dissociated by passing them through a wide-bore 5 ml glass pipette using 10–12 gentle strokes. Non-dissociated ganglia were allowed to settle, and the supernatant was removed and saved. The remaining ganglia were resuspended in 3 ml of Gey's buffer containing 10% horse serum and mechanical dissociation was performed as before. The combined supernatants were filtered through a nylon net (50 μ m pore size) and centrifuged for 10 min at 120 \times g. The preplating procedure used for the neuronal cultures was omitted for the binding studies, because of the low number of non-neuronal cells obtained from chick sympathetic ganglia. Control experiments revealed no significant differences between binding data obtained before and after preplating. The cell pellet was resuspended in Krebs-Ringer HEPES buffer, pH 7.35 (Herrup and Thoenen, 1973), containing 5 mg/ml bovine serum albumin (Sigma) and 0.1 mg/ml horse heart cytochrome C (Sigma). The number of living cells in the suspension was determined by the trypan blue exclusion method.

Binding assays. All binding studies were performed at 4°C. Nonspecific binding was determined by preincubating the cells with a 100–1000-fold excess of unlabeled factor and was subtracted from total binding to obtain specific binding. All values refer to specific binding and are the result of tetraplicate determinations. Binding was terminated by the centrifugation of aliquots of cell suspensions (125 μ l, 350,000–1,500,000 cells/ml) through a two-step sucrose gradient, as described previously (Rodríguez-Tébar and Barde, 1988). Immediately after centrifugation, the tubes were frozen in an ethanol-dry ice bath and the bottoms of the tubes were cut out and counted (bound radioactivity). Free radioactivity was counted from the remaining upper part of the

tubes. The experiments reported here were performed using very low concentrations of 125 I-NT-3, 9×10^{-12} M or less. Together with the gradient centrifugation method, which selects for receptors with slow off-rate, this procedure allows the study of the high-affinity NT-3 binding sites without significant contribution from the numerous low-affinity sites on sympathetic neurons (Godfrey and Shooter, 1985; see Rodríguez-Tébar et al., 1992, for discussion). Affinities and numbers of binding sites were calculated by a linear regression program using a Scatchard transformation of the binding data.

Steady state binding. Cell suspensions were incubated with various concentrations ($1-9 \times 10^{-12}$ M) of radioiodinated NT-3 in a total volume of 525 μ l. The incubation was terminated by centrifugation as described above.

Kinetics of dissociation. A suspension of sympathetic neurons was incubated with 125 I-NT-3 at a concentration of 4×10^{-12} M for 2 hr in order to allow for equilibrium conditions to be reached. Bound radioactivity was measured as described above in octuplicate. A 100-fold excess of unlabeled NT-3 was then added to the suspension and bound radioactivity was determined at various time points in tetraplicate.

Binding in the presence of heterologous ligand. E11 sympathetic neurons were incubated with various concentrations of native heterologous ligand for 60 min, followed by the addition of radioligand. Incubation was continued for another 60 min. Tetraplicate samples were processed for separation of bound radioactivity as described above.

Results

Survival of E11 sympathetic neurons with NT-3

In the concentration range between 0.1 and 1 ng/ml ($3.85-38.5 \times 10^{-12}$ M), NT-3 was found to have no survival activity on E11 sympathetic neurons (Fig. 1). As shown before, the effect of NT-3 on E8 DRG sensory neurons reaches saturation in this range of concentrations (Götz et al., 1992), which corresponds to the occupancy of the high-affinity NT-3 receptor on sensory neurons (Rodríguez-Tébar et al., 1992). At higher concentrations, however, NT-3 clearly elicited a survival response on E11 sympathetic neurons: at 100 ng/ml (3.85×10^{-9} M), the survival response was about half that seen with 1 ng/ml NGF (Fig. 1). These effects of NT-3 are not due to NGF (possibly present as a contaminant), as a monoclonal antibody that fully blocks the NGF response does not reduce the effects seen with NT-3 (Fig. 1). These results also demonstrate the specificity of the anti-NGF monoclonal antibody 27/21 for NGF, when challenged with NT-3 (present results) or BDNF (Rodríguez-Tébar et al., 1990).

High-affinity NT-3 binding sites on E11 sympathetic neurons

Binding studies were then performed using E11 sympathetic neurons and radiolabeled NT-3. High-affinity NT-3 binding sites were detected on these neurons: 1900 ± 200 high-affinity sites per sympathetic neuron were measured with a K_d of $1.6 \pm 0.2 \times 10^{-11}$ M (Fig. 2). As measurements of dissociation rates offer an additional possibility to characterize high-affinity NT-3 receptors, the rate of dissociation of 125 I-NT-3 (used at 4×10^{-12} M) from these neurons was determined (Fig. 3). A value of $6.8 \times 10^{-4} \text{ sec}^{-1}$ was obtained, which is close to the rate measured for the dissociation of NT-3 from its high-affinity receptors on sensory neurons, $4.8 \times 10^{-4} \text{ sec}^{-1}$ (Rodríguez-Tébar et al., 1992). This value is distinctly slower than the rate of dissociation of NT-3 from the LANR ($6 \times 10^{-3} \text{ sec}^{-1}$, Rodríguez-Tébar et al., 1992).

NT-3 and the high-affinity NGF receptors

The observation that high concentrations of NT-3 would support the survival of sympathetic neurons (Fig. 1) suggested that NT-3 might exert its effects on sympathetic neurons using the high-affinity NGF receptors (these cells do not display measur-

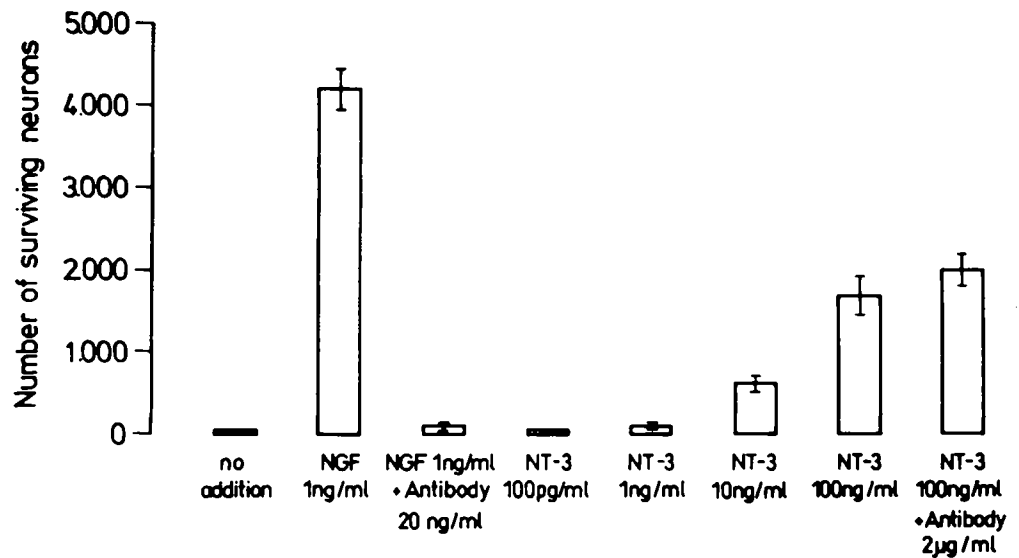


Figure 1. Survival of cultured E11 sympathetic neurons in the presence of high concentrations of NT-3. Sympathetic neurons were isolated from E11 chick lumbar sympathetic chains; 6000 neurons were plated per well and the survival was determined after 72 hr. Results represent the means of three determinations \pm SD.

able levels of high-affinity BDNF receptors; Rodríguez-Tébar and Barde, 1988). In order to test this possibility, binding studies were performed using sympathetic neurons that were incubated with various concentrations of unlabeled NT-3, followed by the addition of labeled NGF at 1.5×10^{-11} M. It was found that 50% inhibition of high-affinity NGF binding was obtained with 2×10^{-10} M NT-3 (or 5.4 ng/ml) (Fig. 4A). These data support the idea that NT-3 can occupy the high-affinity NGF receptor. In the converse experiment, the selectivity of the high-affinity NT-3 receptors on sympathetic neurons was found to be very high when challenged with NGF: 5×10^{-8} M NGF was needed to prevent 50% of NT-3 high-affinity binding (Fig. 4B). This high selectivity is similar to that reported for the high-affinity NT-3 receptors on E8 sensory neurons when challenged with either BDNF or NGF.

Effects of NT-3 on E7 sympathetic cells *in vitro*

We then examined the possibility that the presence of high-affinity NT-3 receptors on E11 sympathetic neurons might indicate that NT-3 has a survival function at earlier stages of development. In order to test this hypothesis, we investigated the effect of NT-3 on neurons isolated from chick E7 sympathetic chains (Fig. 5). For up to 4 d in culture, these cells can survive in the absence of added neurotrophins, but subsequently die, even in the presence of NGF, due to their inability to

differentiate into NGF-dependent neurons *in vitro* (Ernsberger et al., 1989). In fact, these E7 cells lack high-affinity NGF receptors, which can be induced by the addition of retinoic acid to the culture medium (Rodríguez-Tébar and Rohrer, 1991). We found that the presence of NT-3 during the first 3 d *in vitro* did not result in differences in cell numbers compared with cultures without addition of neurotrophins or containing NGF (Fig. 5). However, in the presence of 2 ng/ml NT-3, the sympathetic neurons had more and longer neurites compared to control cultures. After 7 d *in vitro*, $40 \pm 6\%$ of the cells initially plated survived in the presence of NT-3, while $94 \pm 2\%$ of the cells had died in the controls (Fig. 5). Most of the cells surviving in the presence of NT-3 not only were maintained in culture for at least 2 weeks, but also displayed an extensive network of neurites (Fig. 6). In steady state binding assays, E7 sympathetic cells were tested for the presence of high-affinity binding of NT-3. Approximately 2000 binding sites per cell with an affinity similar to that determined for E11 cells were measured (data not shown).

Discussion

High-affinity NT-3 receptors on E11 sympathetic neurons

In terms of both equilibrium binding and rate constants, the high-affinity binding sites for NT-3 on E11 sympathetic neurons are indistinguishable from the high-affinity binding sites pre-

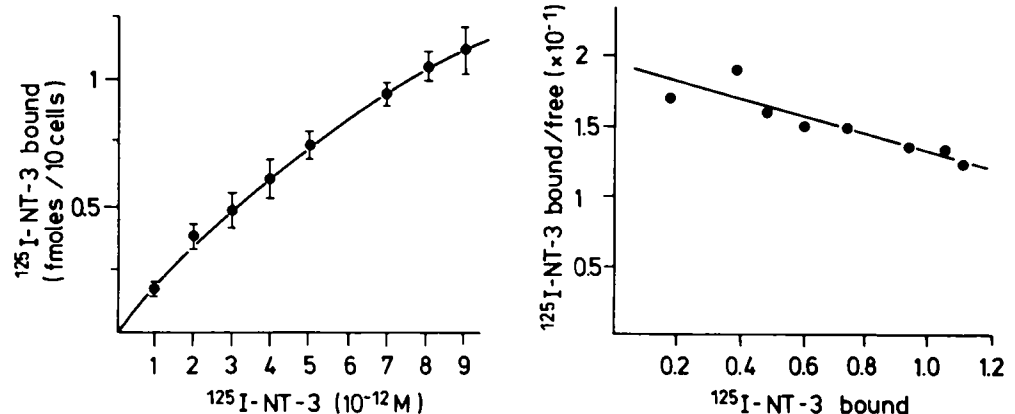


Figure 2. High-affinity binding of $^{125}\text{I-NT-3}$ to E11 sympathetic neurons. The Scatchard transformation revealed 1900 ± 180 sites per neuron with a K_d of $1.6 \pm 0.2 \times 10^{-11}$ M.

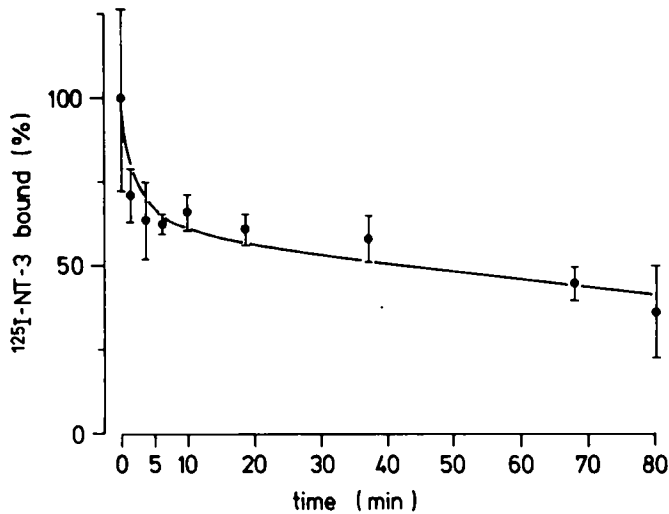


Figure 3. Dissociation kinetics of NT-3 from its high-affinity receptors on E11 sympathetic neurons. ¹²⁵I-NT-3 was used at a concentration of 4×10^{-12} M. The rate of dissociation measured was 6.8×10^{-4} sec⁻¹.

viously described on sensory neurons (Rodríguez-Tébar et al., 1992). The dissociation constant measured here (1.6×10^{-11} M) is also similar to that observed with NGF on sympathetic neurons (3.0×10^{-11} M) and sensory neurons (2.3×10^{-11} M) as well as for BDNF on sensory neurons (0.9×10^{-11} M) for their respective high-affinity receptors (Sutter et al., 1979; Godfrey and Shooter, 1985; Rodríguez-Tébar and Barde, 1988). Furthermore, the high-affinity NT-3 receptors on sympathetic neurons also have the ability to discriminate readily between NT-3 and NGF, a feature common to all high-affinity neuronal neurotrophin receptors characterized so far. In fact, almost identical concentrations (about 5000-fold molar excess) of NGF are necessary to prevent 50% of the binding of NT-3 to its high-affinity receptors on both sensory and sympathetic neurons (Fig. 4B; Rodríguez-Tébar et al., 1992). The number of high-affinity binding sites on E11 sympathetic neurons (1900 per cell) is somewhat lower than the number of receptors on sensory cells (5000 per cell) or the number of NGF receptors (about 4000 per cells) on the same neurons as measured under similar experimental conditions (Godfrey and Shooter, 1985).

In view of these results, it is surprising to observe that NT-3, when used at concentrations that are sufficient to occupy its high-affinity receptor (1 ng/ml corresponds to $2.4 \times$ the K_d), does not promote the survival of E11 sympathetic neurons, although these same cells have the full machinery to display a survival response to another neurotrophin, NGF, as shown in numerous previous studies (see also Fig. 1). This represents the first demonstration of embryonic neurons binding a neurotrophin with high affinity without a survival response. This implies that high-affinity NT-3 receptors exist that are not necessarily linked with the transduction of an *in vitro* neuronal survival response.

The lack of survival response observed with NT-3 on E11 sympathetic neurons prompted us to look for possible effects of NT-3 on younger sympathetic cells. This was all the more interesting as in a recent study, NT-3 was shown to be a mitogen for immature neurons derived from the neural crest, suggesting a biological function for NT-3 prior to the period of naturally occurring neuronal death (Kalchauer et al., 1992). As reported before, cell division still occurs in cultured E7 sympathetic neurons (Ernsberger et al., 1989; Fig. 5), but in the present case,

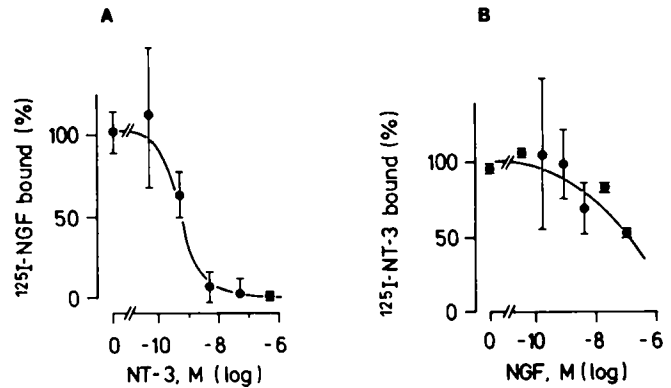


Figure 4. A, Binding of ¹²⁵I-NGF to its high-affinity receptors on E11 sympathetic neurons in the presence of various concentrations of native NT-3. B, Binding of ¹²⁵I-NT-3 to its high-affinity receptors on E11 sympathetic neurons in the presence of native NGF.

the addition of 2 ng/ml NT-3 to E7 sympathetic cultures did not result in enhanced cell proliferation: the number of neurons determined after 4 d *in vitro* did not exceed that found in control cultures lacking neurotrophin or with 2 ng/ml NGF (Fig. 5). However, an effect was observed on the morphology of the cells, which had longer and more profuse neurites than the control cultures. In addition, a proportion of the cells initially plated survived for up to several weeks in cultures containing 2 ng/ml NT-3, but not in control cultures. It is unclear if the cells surviving in the presence of NT-3 represent a subpopulation of early sympathetic neurons prone to become NT-3 dependent *in vivo* or whether these neurons have been influenced in their decision to differentiate into NT-3-dependent neurons by the *in vitro* environment. The simultaneous addition of NGF together with NT-3 did not increase the number of surviving cells (data not shown), indicating that NT-3 is not inducing the capacity of responding to NGF in these cells.

NT-3 and the high-affinity NGF receptor

In several reports describing the *in vitro* effects of NT-3, some discrepancies are apparent with regard to the question of whether or not NT-3 supports the survival of chick sympathetic neurons in culture (compare, for example, Hohn et al., 1990, with Rosenthal et al., 1990). The data reported in the present study suggest an explanation. It seems that already at moderately high concentrations, NT-3 can occupy and utilize the high-affinity NGF receptor. In principle, the idea that the neurotrophins might use each other's high-affinity receptors at high concentrations is not new. Such a case has already been reported using sensory neurons isolated from the nodose ganglion (Rodríguez-Tébar et al., 1990). These placode-derived neurons, while responding to low concentrations of BDNF, are not supported by similarly low concentrations of NGF (nor are they supported *in vivo* by the addition of exogenous NGF; Hofer and Barde, 1988). However, high concentrations of NGF (5 μg/ml, 1.92×10^{-7} M) clearly support the *in vitro* survival of some of these neurons. The suggestion has been put forward that this was due to the occupancy and use by NGF of the high-affinity BDNF receptor. At very high concentrations, NGF can indeed reduce high-affinity BDNF binding on sensory neurons (Rodríguez-Tébar et al., 1990). As shown in this study, when used at low concentrations, NT-3 does not support the survival of sympathetic neurons. However, at higher concentrations, NT-3 does promote their survival, with 100 ng/ml giving about half the re-

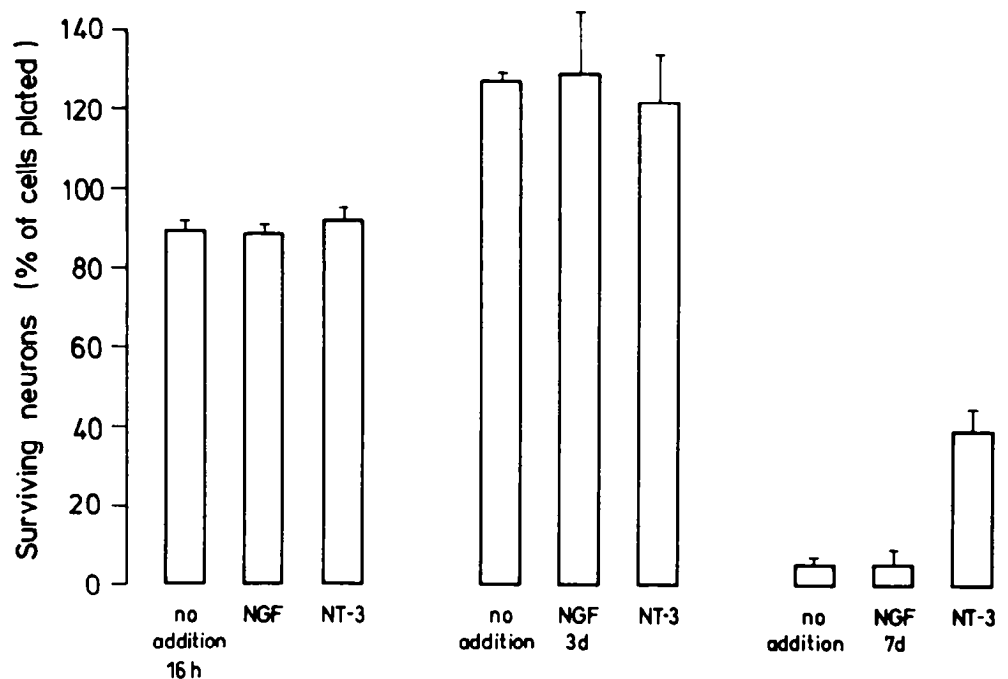


Figure 5. Effect of NT-3 on E7 sympathetic neuronal survival in culture. Sympathetic neurons were isolated from E7 lumbar sympathetic chains; 1500 neurons were plated per well and the number of living cells was counted at the time points indicated. Results are the mean of three determinations \pm SD.

sponse seen with 1 ng/ml NGF. This effect of NT-3 is not mediated by an NGF contamination, because a monoclonal antibody that completely abolishes the biological activity of NGF does not block this response. The observation that the survival effects of NT-3 on these neurons developed already at moderately high concentrations of ligand allowed us to test the prediction that NT-3 would be able to occupy the high-affinity NGF receptor at comparatively lower concentrations (compared with the occupancy of the high-affinity BDNF receptor by NGF). Such was indeed the case: already at 2×10^{-10} M, NT-3 reduces high-affinity binding by 50%. In the converse experiment, considerably more NGF was needed to prevent the binding of NT-3 to its high-affinity sites. This result suggests that in addition to its specific domain responsible for high-affinity, specific binding, NT-3 also possesses a domain able to interact efficiently with the high-affinity NGF receptors on neurons. In this respect, NT-3 differs from BDNF and NGF, which are both unable to interact efficiently with heterologous high-affinity neurotrophin receptors on neurons (Rodríguez-Tébar et al., 1990, 1992; present results).

Molecular nature of the high-affinity NT-3 receptors

NT-3 has been reported to bind to four different receptor molecules expressed on stably transfected fibroblastic cell lines: the low-affinity NGF (or neurotrophin) receptor (LANR; Rodríguez-Tébar et al., 1992) and the tyrosine kinase receptors *trk*, *trkB*, and *trkC* (Cordon-Cardo et al., 1991; Glass et al., 1991; Klein et al., 1991; Lamballe et al., 1991; Soppet et al., 1991). Of these receptors, only the cDNA sequence of the LANR has been elucidated in the chick (Heuer et al., 1990). At present, it is therefore not possible to correlate the expression pattern of the individual receptor candidates with the binding characteristics of NT-3 using chick neurons. Among the *trk* receptors, *trkC* is the most likely candidate to be a specific NT-3 receptor on neurons (Lamballe et al., 1991). Indeed, it is the only *trk* molecule that binds NT-3 with both high affinity and high specificity (Lamballe et al., 1991). These data are in good agreement

with the results obtained with NT-3 in binding studies with sensory neurons (Rodríguez-Tébar et al., 1992). The simplest hypothesis to explain the high-affinity NT-3 binding characteristics found in the present study with sympathetic neurons (similar to those observed with sensory neurons, but without survival response) would be to envisage the expression by E11 sympathetic neurons of *trkC* variants, able to bind NT-3 but not to transduce a signal. Such a case has already been documented for *trkB*, known to exist in rodents with and without a tyrosine kinase domain (Klein et al., 1989; Middlemas et al., 1991). Recent experiments in rodents also indicate the presence of *trkC* variants lacking functional tyrosine kinase domains (L. Parada and P. Tsoulfas, personal communication). When the appropriate tools become available for the chick, the testable prediction resulting from these considerations is that as the development of the chick sympathetic neurons progresses, the expression of full-length *trkC* molecules might decrease to be replaced by other, nonfunctional forms of *trkC*. Our study also indicates that NT-3 can block the binding of NGF to its high-affinity receptors at concentrations substantially lower than that used in any heterologous combinations tested so far. These results are reminiscent of those obtained using *trk* expressed on cell lines: NT-3 has been reported to be a reasonably good agonist of this receptor that mediates primarily the effects of NGF (see Cordon-Cardo et al., 1991). The results (Fig. 1) demonstrating that NT-3 supports the survival of sympathetic neurons (a typical NGF response), but only when used at concentrations beyond the saturation of its own high-affinity receptors, are in good agreement with the results obtained using a *trk* cell line (Cordon-Cardo et al., 1991).

A possible role for nontransducing high-affinity receptors

It is interesting to note that the binding characteristics of high-affinity neurotrophin receptors on neurons described so far resemble the binding properties of high-affinity blocking antibodies, especially with regard to their selectivity (a further example of selectivity of an anti-NGF monoclonal antibody with regard

A



B

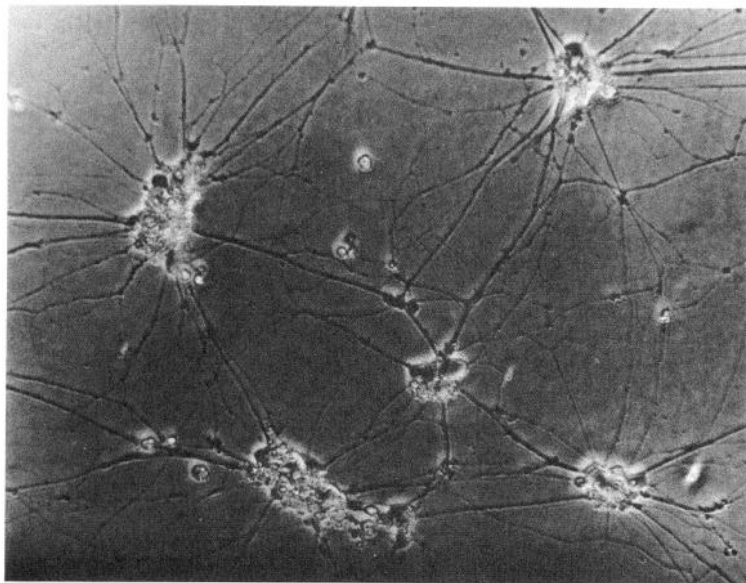


Figure 6. NT-3 promotes the survival and the arborization of E7 sympathetic neurons. Cells were cultured for 7 d in the absence (A) or presence (B) of 2 ng/ml NT-3.

to NT-3 is documented here). Following on this analogy with the example of the sympathetic neurons used in this study, it could be envisaged that as these neurons mature, they decorate their surfaces with nontransducing forms of *trkC* that neutralize (after selective binding and internalization) NT-3, when its action is no longer required. This is reminiscent of one of the suggestions made regarding the possible role of the truncated form of *trkB* as a "clearance receptor" (Klein et al., 1989). NT-3 might be required for some aspect of the early differentiation of sympathetic neurons, like axonal growth and arborization (see Results), but this action might no longer be desirable on specific sets of axonal terminals later in development. Such a strategy would not require the expression of the NT-3 gene to be stopped, which might not be appropriate if other NT-3 responsive structures in the vicinity, for example, sensory nerve endings, still require this protein. In addition, our data also suggest a reason why such a strategy might have evolved, as

opposed to the other possibility of simply ceasing NT-3 receptor expression altogether. As discussed, high concentrations of NT-3, if allowed to build up *in vivo*, could result in activating the high-affinity NGF receptors. Maintaining a selective removal system that prevents the buildup of neurotrophins might be an important function of the nontransducing forms of high-affinity neurotrophin receptors in order to prevent interaction with heterologous receptors.

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