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Functional Roles of Neurotrophin 3 in the Developing and Mature Sympathetic Nervous System

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Abstract

Nerve growth factor (NGF) is a potent regulator of sympathetic neuronal function in both developing and adult animals. This article reviews the evidence published in recent years indicating that another member of the NGF family, neurotrophin 3 (NT3), plays both a complementary and overlapping role in the development and maturation of sympathetic neurons. In migratory neural crest cells, expression of the high-affinity receptor, trkC, and promotion of mitosis by NT3 suggest an involvement in gangliogenesis, since sympathetic neuroblasts express both NT3 and trkC and require NT3 for their proliferation, differentiation, and survival, it has been proposed that the factor acts at this developmental stage as an autocrine or paracrine factor. However, NT3 also acts in parallel with NGF to promote the survival of postmitotic neurons during late development. Both trkC and trkA are expressed in sympathetic neurons and function as high-affinity receptors for NT3. NT3 is synthesized in sympathetic effector tissues and the endogenous factor is retrogradely transported to accumulate within the cell soma. Thus, in addition to its role in the differentiation of sympathetic neurons, NT3, like NGF, is also an effector tissue-derived neurotrophic factor for these neurons in maturity.

Index Entries: Neurotrophin; sympathetic neurons; development; neurotrophic factors; trk; differentiation.

Introduction

The neurotrophin family consists of four structurally related basic polypeptides containing 115–130 amino acid residues. Nerve growth factor is the prototypic member of the family, promoting the survival of sympathetic and sensory neurons during development. Other members of the family are brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and NT4/5 (Korsching, 1993). These molecules form homodimers under physiological conditions, share 50–60% structural homology and display overlapping but distinct

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biological functions, including promoting the survival, differentiation, and proliferation of a number of neuronal populations (Thoenen, 1995). Interestingly, in parallel with the discovery of these neurotrophins, a family of tyrosine kinase receptors named trkA, trkB, and trkC have been cloned and identified as the high-affinity receptors for the neurotrophin family. It is now generally agreed that trkA is the high-affinity receptor for NGF, trkB for BDNF and NT4, and trkC for NT3 (Barbacid, 1993). Neurotrophins bind to the trk receptors with high-affinity, triggering their autophosphorylation and evoking a cascade of second messengers. Despite the relative specificities in the action of each neurotrophin on their respective receptors, crosstalk is apparent. For example, NT3 can also activate trkC and trkB, and BDNF activates trkC, with lower potencies. All neurotrophins share a common affinity for the low-affinity NGF receptor, p75. The function of p75 is still a subject of debate. Although it is generally agreed that this receptor affects neurotrophin sensitivities it is not essential for the transduction of most neurotrophin signals (Verdi et al., 1994).

In the short time since the discovery of the neurotrophins and their receptors, significant progress has been made on their functional roles in the development of the nervous system and is well described in a number of recent reviews (Levi-Montalcini, 1987; Barde, 1989; Bothwell, 1991; Chao, 1991; Thoenen, 1991, 1995; Barbacid, 1993; Korsching, 1993; Gotz and Schartl, 1994; Klein, 1994). NT3, like the other neurotrophins, demonstrates multiple functions on neuronal development. It acts as a survival and differentiating factor on a number of neuronal populations, including sensory, sympathetic, and motor neurons in the peripheral nervous system (PNS) and several neuronal populations throughout the central nervous system (CNS). In this article, we have focused on the progress made in understanding the role NT3 plays both in the developing and mature sympathetic nervous system, since its identity was revealed six years ago.

NT3 Is a Differentiation and Survival Factor for Sympathetic Neuroblasts

Subsets of premigratory and migrating neural crest cells express trkC-mRNA very early in the development of the PNS (Kahane and Kalcheim, 1994; Yao et al., 1994; Zhang et al., 1994). It is expressed only in those neural crest populations that possess neurogenic potential, suggesting that a subpopulation of neural crest cells expressing functional trkC receptors require NT3 for their development before they migrate to their destination (Litenion et al., 1995). That activation of this trkC is required for neuronal differentiation has been tested in vitro where the presence of NT3 is required for neurogenesis. NT3 acts as a mitogen on cultured neural crest cells and, in the presence of somites, promotes their proliferation (Kalcheim, 1992). Consistent with this mitotic effect is the expression of NT3-mRNA in somites as early as embryonic d (E) 1–2 of chick and E11–12 of rat embryos (Maisonpierre et al., 1990; Yao et al., 1994). However, in contrast to these studies in vitro, application of exogenous NT3 to chick embryos between E3 and 6, but not later, significantly reduces the number of sensory neurons by retarding the proliferation of neuroblasts (19). These studies suggest that NT3 regulates gangliogenesis during development by controlling the proliferation of neural crest cells.

During genesis of sympathetic neurons, sympathetic neuroblasts in chick, mouse, and rat express mRNAs for both NT3 and *trkC* (Ernfors et al., 1992; Schechterson and Bothwell, 1992; Kahane and Kalcheim, 1994; Zhang et al., 1994). Nonneuronal cells in the sympathetic ganglia also synthesize NT3-mRNA, which is upregulated by platelet-derived growth factor, ciliary neurotrophic factor and neuregulin (Verdi et al., 1996). Sympathetic neuroblasts enhance the production of NT3 by nonneuronal cells through the secretion of neuregulin (Verdi et al., 1996). The function of NT3 in these cells has been well studied by several groups that have shown that, in cultured sympathetic neuroblasts taken from rats

at E14.5–15.5, NT3 increases the number of mature sympathetic neurons by promoting the survival of proliferating sympathetic neuroblasts and stimulating their differentiation (Direen et al., 1992; DiCiccoBloem et al., 1993). The ratio of cells incorporating thymidine incorporation to the total number of surviving neurons in the presence of NT3 was comparable to the control, suggesting that NT3 has no significant effect on the mitosis of sympathetic neuroblasts (DiCicco-Bloom et al., 1993). It also appears that this survival effect of NT3 does not last long. Culture experiments have suggested that the sensitivity of sympathetic neurons to NT3 for survival declines during late developmental stages, so that only a small population of rat neonatal sympathetic neurons respond to NT3, even when administered in high doses (Birren et al., 1992; Lee et al. 1994). During this period, these postmitotic sympathetic neurons become dependent on NGF for their survival both in vitro and in vivo (Levi-Montalcini, 1987). This reciprocal change in dependence on neurotrophins for their survival is mirrored by changes in receptor expression. TrkC-mRNA levels in the superior cervical ganglion of E15 embryos are 10 times higher than in ganglia from newborn rats (Birren et al., 1992; DiCicco-Bloom et al., 1993; Verdi and Anderson, 1994; Black et al., 1995). Interestingly, this dynamic decline in trkC-mRNA levels is accompanied by an increase in the expression of mRNAs, first for trkA and then for p75 (Verdi and Anderson, 1994; Verdi et al., 1995) (*see* Fig. 1). NT3 is required for this receptor upregulation in the newly generated sympathetic neurons (Verdi and Anderson, 1994). However, the concentration of NT3 required for the upregulation of trkA is 100 times higher than for the survival of these sympathoblasts, so whether this represents a physiological role requires further elucidation. A correlation also has been seen between the increasing levels of NT3-mRNA from E14–17 with the appearance of trkC-mRNA (Verdi et al., 1995). *In situ* hybridization studies suggest that both neuronal and nonneuronal cells synthesize NT3-mRNA in the sympathetic ganglia during this period (Yao et al., 1994; Zhang et al., 1994; Verdi et al., 1996), but only in nonneuronal cells in adult animals (Wetmore and Olson, 1995). Up-regulation of trkA by NT3 at this stage of development may be related to its ability to promote cell–cycle arrest, since ciliary neurotrophic factor (CNTF) and antimitotic agents, which induce this effect, also upregulate trkA-mRNA (Verdi and Anderson, 1994).

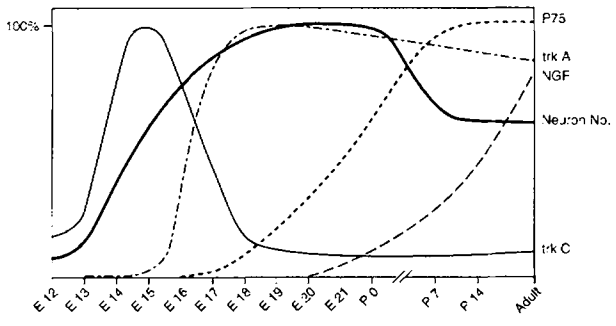

Fig. 1. A schematic diagram with relative scales depicting developmental profiles of proteins expressed by rat sympathetic neurons. The data used for this diagram were taken from several published studies (Wright et al., 1983; Rubin, 1985; Korsching and Thoenen, 1988; Zettler and Rush, 1993; Verdi and Anderson, 1994).

These observations support the idea that NT3 acts locally within ganglia as a differentiation factor to upregulate trkA through an autocrine or paracrine mechanism. Thus, early postmitotic neurons become responsive to the survival effects of NGF, which is derived principally from effector tissues. In addition to its differentiative role, it appears that NT3 at this stage acts as an interim survival factor rather than a peripherally derived factor (Verdi and Anderson, 1994), since no connection of these cells to their targets has been established during the period of their greatest sensitivity to NT3. Furthermore, consistent with these studies in vitro, deletion of the NT3 gene results in a severe sympathectomy in newborn mice (Rosenthal et al., 1990; Ernfors et al., 1994a; Farinas et al., 1994; Reichardt et al., 1995; Elshamy et al., 1996), although these studies did not distinguish between the loss of sympa-

thetic neurons resulting from death of sympathetic neuroblasts or from postmitotic neurons. Further studies on these mutant animals, however, have shown that endogenous NT3 is required for the survival of the sympathetic neuroblasts, since excessive apoptosis occurs in the superior cervical ganglia (SCG) of $NT3^{-/-}$ mice during early development, and the number of neurons in the ganglia compared with the wild-type decreases progressively from E12.5 (Elshamy et al., 1996). In addition, there is no further reduction in neuronal numbers in the SCG from E17 to postnatal d (P) 7, indicating endogenous NT3 has no effect on the survival of sympathetic neurons during naturally occuring cell death in these mutant mice. However, it is important to be cautious when drawing conclusions about the normal role of NT3 from mutant animals, since the absence of NT3 for the entire developmental period can lead to secondary or compensatory changes in the neuronal phenotype.

Evidence That NT3 Is a Survival Factor for Mature Sympathetic Neurons

It is interesting to note that the first group of papers in 1990 characterizing the NT3 gene and recombinant protein all described survival effects of NT3 on sympathetic neurons during the period of naturally occurring cell death in different species (Ernfors et al., 1990; Hohn et al., 1990; Maisonpierre et al., 1990b; Rosenthal et al., 1990). These studies suggested that sympathetic neurons require NT3 for their survival after their processes reach effector tissues and during the innervation period. More recent studies, however, indicated that these neurons respond poorly to NT3 in culture. Whether NT3 functions to prevent naturally occurring cell death in this late developmental period is an important question, since this role has been well established for NGF. Accumulating evidence now suggests that NT3 acts in parallel with NGF as a survival factor for sympathetic neurons not only during late development, but also in adult animals.

Exogenous NT3 is known to bind specifically to mature sympathetic neurons (Rodriquez-Tebar, 1992; Dechant et al., 1993), to be retrogradely transported to neuronal perikarya of adult neurons (DiStefano et al., 1992), and to support the survival of cultured sympathetic neurons taken from neonatal mice (Lee et al., 1994; Davies et al., 1995; Elshamy et al., 1996). Although the level of trkC-mRNA in sympathetic neurons is low in neonatal and adult animals compared with early developmental stages (Verdi and Anderson, 1994; Black et al., 1995), the presence of both trkC-and trkA-mRNA and protein is readily detectable throughout the entire life of the animal (Dixon and McKinnon, 1994; Wetmore and Oson, 1995; Zhou et al., 1996). Immunohistochemical studies clearly demonstrate that immunoreactivity for both trkA and trkC is present in most neurons of the superior cervical ganglion of neonatal and adult rats (Fig. 2). Furthermore, high levels of NT3-mRNA can be detected in sympathetic targets, such as iris, heart, kidney, and blood vessels, in developing and adult rat (Maisonpierre et al., 1990a; Ernfors et al., 1992; Schecterson and Bothwell, 1992; Zhou et al., 1996).

Currently two independent studies in neonatal rats and mice have clearly demonstrated that NT3, like NGF, is a survival factor for these fully differentiated sympathetic neurons (Zhou and Rush, 1995a; Elshamy et al., 1996). Using an antiserum to NT3, we have demonstrated that most sympathetic neurons require endogenous NT3 for their survival in the first two postnatal weeks (Zhou and Rush, 1995a). Neuropeptide Y (NPY) immunoreactive (ir) neurons are particularly susceptible to the NT3 antiserum treatment. Other neuronal markers, such as tyrosine hydroxylase and the low-affinity neurotrophin receptor, p75, are also affected. Sympathetic innervation in target tissues is significantly reduced by the neutralization of NT3. Since NT3 acts during the same period as NGF to support most sympathetic neurons, it is reasonable to conclude that these

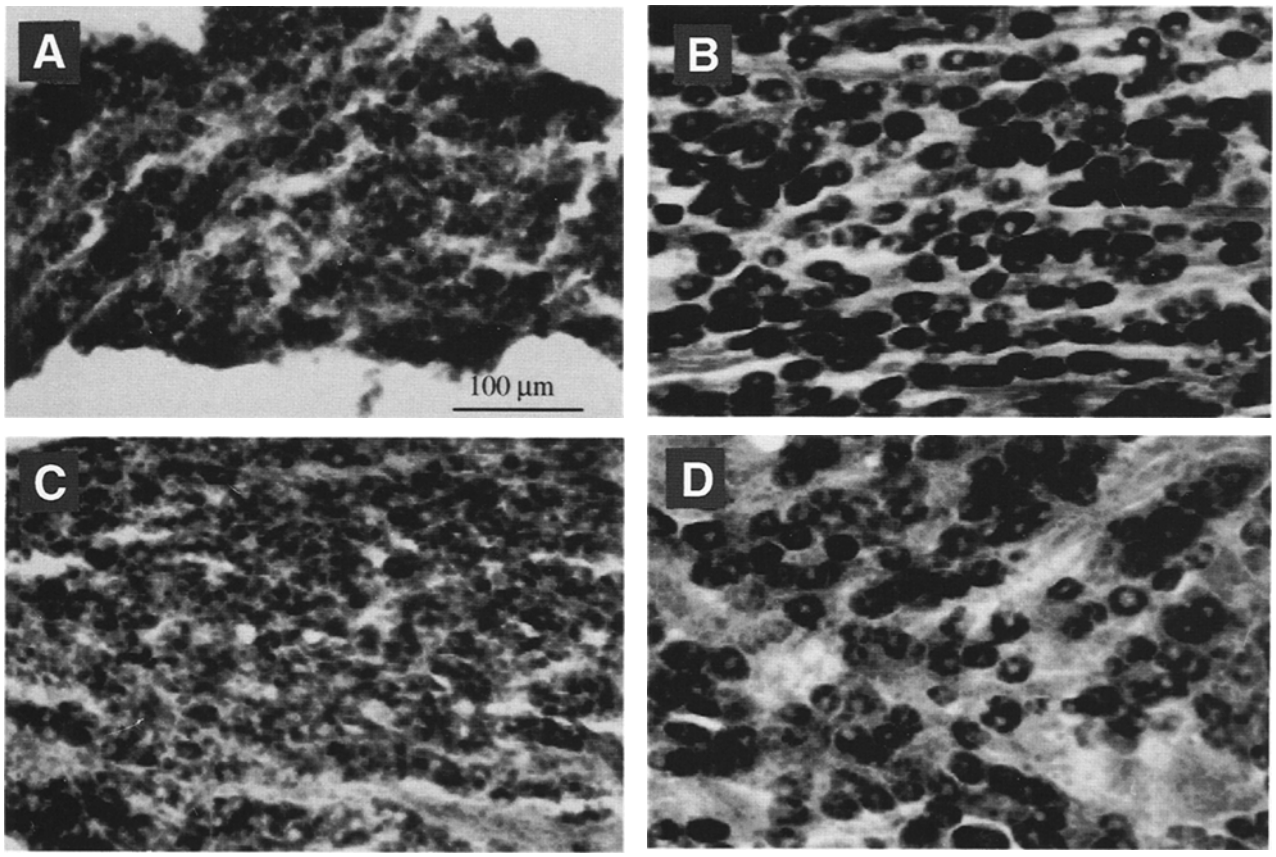

Fig. 2. Micrographs of trkA-ir and trkC-ir sympathetic neurons of SCG in 1-wk-old and adult rats. Polyclonal antibodies to trkA were from L. Reichardt and raised against extracellular domain of trkA in a rabbit (Sobreviela et al., 1994). Polyclonal antibodies to trkC were raised against a peptide sequence of the intracellular domain of trkC (Santa Cruz Biotechnology, Santa Cruz, CA). **(A,B)** trkA-ir sympathetic neurons in the SCG of 1-wk-old and adult rats, respectively; **(C,D)** trkC-ir sympathetic neurons in the SCG of 1-wk-old and adult rats, respectively.

neonatal neurons require two factors for their survival. This appears to be the first clear example of the reliance of a single population of neurons on a simultanenous supply of two factors.

The second line of evidence comes from several groups that have shown, using $NT3^{-/-}$ mutant mice, that NT3 is required for the survival of sympathetic neurons (Elshamy et al., 1996). In these animals, sympathetic neurons fail to innervate selective organs (Elshamy et al., 1996). This deficit can be reversed by injection of exogenous NT3, supporting the proposal that in postnatal animals, NT3 is normally derived from effector tissues and is required by sympathetic neurons for the innervation process. It is not known, however, why sympathetic neurons from neonatal mutants do not commit to a naturally occurring cell death, which is in contrast with the antibody neutralization experiments. It may be that compensatory effects (e.g., upregulation of NGF synthesis) occurs in mutant mice. Alternatively, abnormal elimination of 50% of all sympathetic neurons before birth may allow survival of the remaining neurons by normal levels of NGF from intact effector tissues. Thus, the proposal, based largely on data derived

from culture studies (Davies, 1994), that sympathetic neurons switch their dependence from NT3 to NGF appears not to occur in vivo. Thus, the conflicting results from studies in vitro and in vivo suggest that the underlying mechanisms for the function of neurotrophins may be different in these different environments. This suggestion is further supported by a recent observation showing NT3 inhibits proliferation of neural crest cells in vivo (Ockel et al., 1996), conflicting with the hypothesis and observation that NT3 promotes mitosis of these cells in vitro (Kalcheim et al., 1992).

A recent finding that helps to resolve this apparent conflict is that the expression of trkC and responsiveness to NT3 by sympathetic neurons in culture decline rapidly (within 4 hours), but can be maintained by the addition of retinoic acid and bone morphogenetic protein 2, a member of the transforming growth factor superfamily. It is also known that retinoic acid induces the NGF-dependent survival response and the expression of high-affinity receptors in immature chick sympathetic neurons (Rodriquez-Tebar and Rohrer, 1991). Interestingly, retinoic acid can upregulate the expression of trkB, inducing a dependence on BDNF by cultured sympathetic neurons, which do not normally express this receptor and are not sensitive to BDNF. In addition, a low concentration of NGF in vitro can maintain the expression of trkC in neonatal sympathetic neurons and responsiveness to NT3 (Belliveau et al., 1995). These studies suggest that expression of trkC and responsiveness of sympathetic neurons to NT3 are critically dependent on extrinsic factors. Alternatively, NT3 may act through different pathways in vivo, for example, through both trkC and trkA receptors, to promote neuronal survival and other responses, particularly, since trkA has two splice variants varying by an 18-bp exon in the extracellular domain (Barker et al., 1993), the larger variant exhibiting a higher sensitivity to NT3 without altered sensitivity to NGF when expressed in PC 12 cells (Clary and Reichardt et al., 1994).

Is It the Total Level of Neurotrophic Support by NGF and NT3 That Is Crucial for the Survival of Sympathetic Neurons?

It is well recognized that NGF is a survival factor for sympathetic neurons (Levi-Montalcini, 1987). Therefore, it is surprising that NT3 acts on these same neurons in parallel with NGF, apparently performing the same function. The fact that a neuron requires the simultaneous supply of two factors for survival raises a number of questions. For example, are both factors essential for the survival, or is it the total amount of trophic support that is critical? Do NT3 and NGF act on the same second messenger pathways? Are NGF and NT3 derived from the same or different peripheral effector tissues? If they are synthesized in the same cells, do they form NT3/NGF heterodimers? Is NT3, like NGF, required for the functional maintenance of sympathetic neurons in adult animals? To address some of these questions, a series of experiments was performed in neonatal rats, using a combination of specific antisera and exogenous factors. Exogenous NT3 was found to rescue sympathetic neurons from death induced by depletion of endogenous NGF with its antiserum. Conversely, exogenous NGF prevented sympathetic neuronal death induced by immunologically blocking endogenous NT3. One difference between the action of NGF and NT3 was uncovered when exogenous NGF or NT3 was given to intact neonatal rats. NGF induced a hyperplasia of the sympathetic ganglia by preventing sympathetic neuron death, whereas NT3 was unable to achieve this rescue (Tafreshi et al., 1996). In contrast, the same dose of exogenous NGF given to NT3-deprived rats did not increase the total number of neurons in the ganglia. These results suggest that for the survival of sympathetic neurons, the total amount of neurotrophic support from their effector tissues is more important than any individual factor.

The molecular mechanisms underlying these interactions are not clear. It is known that

NT3 can bind to and activate both trkC and trkA in nonneuronal fibroblast cells (Lamballe et al., 1991; Clary and Reichardt 1994), although trkC is the preferable receptor. In addition, NT3 activates phosphorylation of both trkC and trkA in sympathetic neurons and PC12 cells in the presence of a low concentration of NGF. Using sympathetic neurons from $trkC^{-/-}$ mutant mice in vitro, Davies and his colleagues showed that their sensitivities to NT3 are similar to that for NGF. However, no response to NT3 can be observed if these neurons are taken from mice with the double $trkA^{-/-}$ $trkC^{-/-}$ mutation. It would be interesting to know whether the survival response of sympathetic neurons to NT3 is impaired in the $trkA^{-/-}$ mutant, particularly, since it is known that a reduced suvival response of trigeminal sensory neurons to NT3 is closely correlated with the reduction in the number of copies of both trkC and trkA genes. The question of whether trkA is a functional receptor for NT3 in vivo has not been resolved. NT3 and trkC gene deletion experiments strongly suggest that NT3 can signal through alternate receptors. Deletion of the trkC gene only induces death of about 20% of spinal sensory neurons and has no effect on neuronal number in sympathetic ganglia (Klein et al., 1994). In contrast, deletion of the NT3 gene results in more severe neuron loss in both dorsal root (55–78% loss) and sympathetic (50% loss) ganglia (Ernfors et al., 1994; Farinas et al., 1994; Reichardt et al., 1995). Recently, we have found that both trkC and trkA in mesenteric arteries, kidney and adrenal gland are constitutively phosphorylated and increased in both receptors in neonatal rats in response to the injection of either exogenous NT3 or NGF. Constitutive phosphorylation of trkC is reduced by pretreatment of rats with NT3 antiserum. These results suggest that both trkC and trkA are functional receptors for NT3 in vivo. A number of studies have shown that heterodimers can be artificially produced with recombinant proteins (Radziejewski and Robinson, 1993; Jungbluth et al., 1994). To date, there is no evidence for the existence of heterodimers in the intact animal, but, since arterial smooth muscle cells synthesize both NGF and NT3 (Scarisbrick et al., 1993), it is reasonable to speculate that an NGF/NT3 heterodimer may exist in this system.

Evidence That NT3 Is a Retrogradely Acting Neurotrophic Factor Derived from Sympathetic Effector Tissues

It is well known that removal of effector tissues in developing animals results in an excessive death of innervating neurons (Hamburger and Levi-Montalcini, 1949; Oppenheim, 1989). Death of sympathetic neurons in embryonic chick induced by limb bud removal cannot be completely reversed by exogenous NGF (Saltis and Rush, 1995), suggesting the involvement of additional factors in this process. In addition, it has been shown by injection of NGF antiserum to neonatal rats that sympathetic neurons in prevertebral ganglia are less sensitive to NGF than those within the paravertebral chain (Zaimis et al., 1965). Indirect evidence suggests that NPY-ir in sympathetic neurons is regulated by factors distinct from NGF (Cowen, 1993). Injury to the sensory innervation of tooth pulp induces NGF-sensitive sproutings from sensory fibers, but not from NPY-ir sympathetic fibers (Edwall et al., 1985; Oswald and Byers, 1993).

Our recent studies have demonstrated that NT3, like NGF, derives from effector tissues to act as a neurotrophic factor for mature sympathetic neurons (Zhou et al., 1996). Using an immunohistochemical technique, most sympathetic neurons from neonatal and adult rats have been shown to express immunoreactivity for both trkA and trkC (Fig. 2). Accordingly, the majority of sympathetic neurons are immunoreactive for both NGF and NT3. The immunoreactivities of one but not the other factor are lost by in vivo absorption with the respective antisera (Zhou et al., 1994, 1996). As has been shown for NGF, NT3-ir accumulates as early

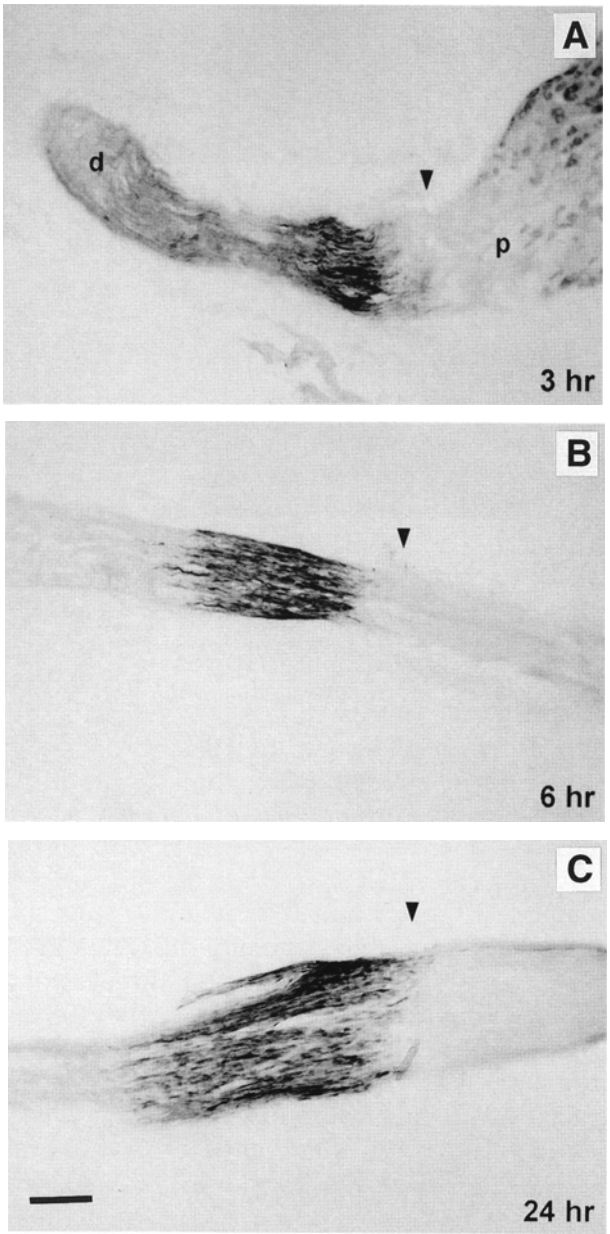

Fig. 3. Micrographs showing retrograde transport of NT3-ir in the rat internal carotid nerve. The internal carotid nerve was crushed with a pair of fine forceps for 30 s, and rats were allowed to survive for different periods of time. The nerves were processed for NT3 immunohistochemistry as described previously by Zhou and Rush (1993b). NT3-ir accumulated on the distal, but not proximal, side of the lesion **(A)** 3, **(B)** 6, and **(C)** 24 h after crushing. Note that NT3-ir was also localized to sympathetic neurons 3 h after lesion, which reduces with time to near background levels. Scale bar in (C) indicates 100 μm.

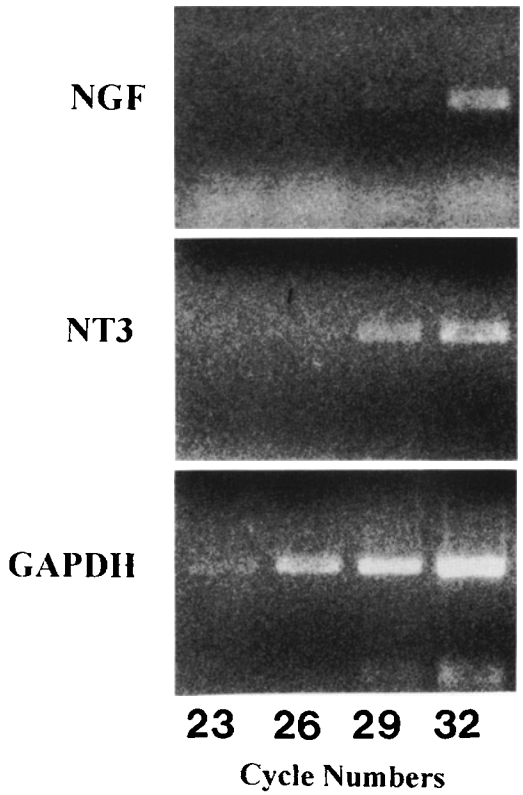

Fig. 4. Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay of NGF-and NT3-mRNAs in the mesenteric arteries of 1-wk-old rat. Total RNA was extracted using RNA extraction reagents from Advanced Biotechnologies under the manufacturer's instruction with some minor modifications. Extraction solution is based on high-molar guanidine salt and urea mixed with phenol and detergents. RT-PCR of the NGF-mRNA, NT3-mRNA, and a housekeeping enzyme glyceraldehyde-3 phosphate dehydrogenase (GAPDH) was performed using the same sample. GAPDH was used as an internal control. As shown in this figure, the level of NT3-mRNA was severalfold higher than that of NGF-mRNA in this sample. This result was reproducible in three additional samples.

as 3 h after operation on the distal, but not proximal, side of the lesioned internal carotid nerve, indicating endogenous NT3 is retrogradely transported by sympathetic neurons (Fig. 3). NT3-mRNA is abundantly expressed in many sympathetic effector tissues, including mesenteric arteries, heart atria and ventricles, salivary gland, and kidney, but not in signifi-

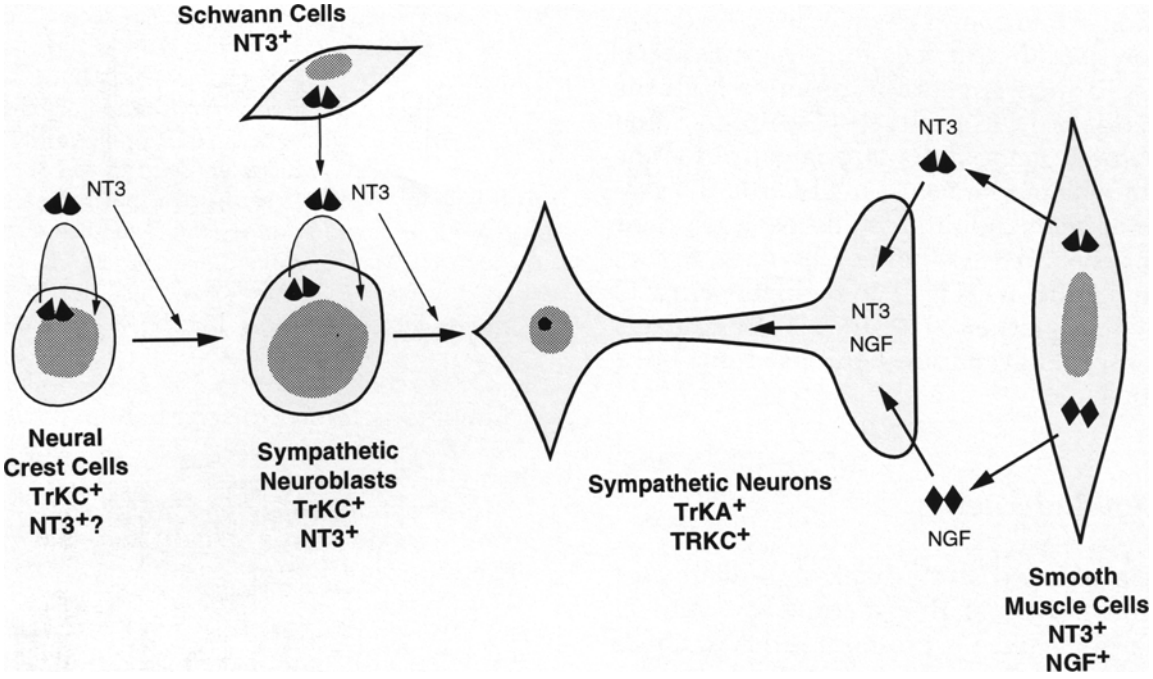

Fig. 5. Schematic diagram showing roles of NT3 in development of sympathetic neurons. Expression of the functional receptor, trkC, in migrating neural crest cells suggests NT3 may play a role in neurogenesis. Expression of NT3 and trkC in sympathetic neuroblasts and nonneuronal cells implies an autocrine and paracrine mechanism operating within sympathetic ganglia. NT3 acts as a survival factor for proliferating sympathoblasts and promotes differentiation and dependence on NGF and NT3 for survival. After axons reaching their effector tissues, postmitotic neurons depend on effector tissue-derived NGF and NT3 for survival and functional maintenance.

cant amounts in the superior cervical ganglia of adult rats (Maisonpierre et al., 1990a; Ernfors et al., 1992, 1994b; Schecterson and Bothwell, 1992, 1994; Scarisbrick et al., 1993; Zhou et al., 1996). The levels of NT3-mRNA in the mesenteric arteries appear higher than for those of NGF-mRNA (Fig. 4) but estimation of protein levels is needed to confirm this. In mesenteric arteries and submaxillary glands, the NT3-mRNA is upregulated at a time that corresponds to the innervation period (Zhou et al., 1996). In the mesenteric artery, it is likely that only the sympathetic nerves utilize the NT3, since the sensory neurons innervating viscera do not contain NT3 (Zhou and Rush, 1995b). Cutaneous and muscle afferents are the only sensory neurons that utilize endogenous NT3, which corresponds to their expression of trkC (Zhou and Rush, 1995b; Chen et al., 1996). Estimation of NT3 protein in effector tissues has only just begun, but is suggestive of levels similar to that of NGF (Katoh-Semba et al., 1996). In some tissues, such as pancreas, kidney, and spleen, NT3-mRNA levels appear much higher than those of NGF-mRNA (Korsching and Thoenen, 1983; Katoh-Semba et al., 1996). However, histological identification of NT3-ir indicates a wider role for this protein in that its localization in some of these tissues does not correlate with the known innervation (Zhou and Rush, 1993a; Katoh-Semba et al., 1996). Nevertheless, the high levels of NT3-mRNA in sympathetic effector tissues, such as arteries and salivary glands, is consistent with the dense sympathetic innervation of these tissues.

In summary, accummulating evidence indicates that NT3 plays an important role throughout the entire life cycle of the sympathetic neuron. This sequential and continuing action of NT3 is schematically depicted in Fig. 5. It

may first act, in concert with other growth factors, on neural crest cells during their migration. NT3 then appears to promote both the differentiation and survival of the proliferating sympathetic neuroblasts through an autocrine or paracrine mechanism. Finally, after the processes of the postmitotic neurons reach their end organs, NT3 synthesized by these tissues acts in parallel with NGF to regulate neuronal survival. Whether NT3, like NGF, controls other aspects of mature neuronal function is unknown.

Acknowledgments

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