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[\IEUROTROPHII\1S: NEURAL ANTIAPOPTOTIC MOLECULES WITH NEURITE GROWTH-PROMOTING PROPERTIES

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SUMMARY

• *Neurotrophins are a family of small proteins that bind to and activate the members of receptor protein tyrosine kinase family. They also bind the low-affinity neurotrophin receptor (p75). Neurotrophins have a broad spectrum of biological functions in several tissues, but they are best studied in the developing nervous system. Neurotrophins are secreted from cells of many tissues, picked up by terminals of the competent neurons innervating these tissues, and transported to the neuronal perikarya. In developing neurons, this leads to neutralization of the naturally occurring neuronal death program and thereby regulates the density of tissue innervation. In neurobiology, the term "trophic" actually means antiapoptotic, rather than nutritional. In addition, neurotrophins can induce neurite outgrowth and direct the course of the neurites.*

IHTRODUCTIOM

• The observations that peripheral tissues have positive influence upon the neurons that innervate them were made by Ross Harrison, Sam Detwiler and Victor Hamburger in the beginning of the 20th century. They demonstrated that limbs transplanted ectopically in amphibian embryos were correctly innervated by neurons that normally do not innervate the limbs, whereas dorsal root ganglia (DRG) innervating the ectopic limbs were hyperplastic (1,2). Conversely, extirpation of the forelimb from salamander and chick embryos led to hypoplasia of the DRG neurons and motor neurons that would normally innervate this limb. These findings led to formulation of the neurotrophic concept: each tissue controls its own nerve center by sending stimuli to the nervous system (3). The word "trophic" is derived from Greek *"trophe"* meaning nourishment or taking up of nutrients. In its origin, the neurotrophism implies that target tissues feed the neurons that innervate them.

The nature of the signals the tissues send to the neurons remained unknown until 1948, when Elmer Bueker and Rita Levi-Montalcini accidentally discovered that the graft of mouse sarcoma 180 tissue in the body wall of the chick embryo secreted some factors that induced massive innervation of the sarcoma by sensory and sympathetic axons (4). Moreover, the sensory and sympathetic ganglia contributing to these axons were abnormally large in volume suggesting that the sarcoma factors had neurotrophic properties (5). The subsequent accidental discovery that snake venom contains the same neurotrophic activity, led to the finding that mouse salivary gland, an organ homologous to the snake venom gland, is an extraordinarily rich source of the neurotrophic substance, thus enabling Stanley Cohen to purify chromatographically a protein from the mouse salivary gland homogenate (6). The protein was named nerve growth factor (NGF) just for its effect on the growth of nerve fibers from embryonic sensory and sympathetic ganglia.

The biological roles of NGF were extensively studied and will be described in the following sections. It appeared that NGF affected only few neuronal populations, whereas many other neurons did not respond to NGF in any way, but were still trophically dependent on their target tissues. The search for putative other neurotrophic factors was, however, hampered by the absence of such a rich source as the mouse salivary gland had been for the NGF. Therefore, it took several years of hard work, when a second neurotrophic factor was described.

In early 1970-s, a neurotrophic activity, biologically and immunologically distinct from NGF, was discovered in the conditioned medium of a C6 glioma cell line (7). Subsequently, the same activity was found in brain extracts and conditioned media of brain cells (8). It took several years of laborious work in the laboratory of Yves-Alain Barde and Hans Thoenen, where pig brain extracts were chromatographically fractionated and assayed on chick DRG neurons for survival-promoting effect, a new neurotrophic factor called brain-derived neurotrophic factor (BDNF) was purified to homogeneity (9).

cDNA for both NGF and BDNF were cloned and sequenced (10-12), and the factors were shown to be highly homologous. Additional neurotrophic factors were now searched by polymerase chain reaction using primers from the areas conserved in NGF and BDNF. In 1990, six groups independently cloned a new neurotrophic factor homologous to NGF and BDNF, that was called neurotrophin-3 (NT-3) (13-18). By the same strategy, a fourth factor called neurotrophin-4 (NT-4) was isolated from *Xenopus laevis* (19), followed by isolation of similar sequences from rat and human genome called neurotrophin-5 (NT-5) (20). NT-5 may be a mammalian paralog of amphibian NT-4, but this is not generally accepted, so in many studies these two are referred to as NT-4/5. In 1994, neurotrophin-6 (NT-6) was cloned from the platyfish *Xiphophorus maculatus* (21), but its homologs in higher vertebrates have not been reported.

The five neurotrophic factors constitute a family called neurotrophins (NTF). All NTF have similar biological effects on different, but partially overlapping neuronal populations. For example, DRG and trigeminal ganglia contain subpopulations of neurons sensitive for each of the four NTF, whereas nodose and geniculate ganglion neurons respond mostly to BDNF and sympathetic neurons mostly to NGF. Statoacoustic ganglion neurons are maintained by BDNF and NT-3 but not by NGF or NT-4, whereas spinal cord motor neurons respond to BDNF, NT-3 and NT-4 *in vitro.*

In the following, structure, receptors and classical functions of NTF in the development of mammalian or avian nervous system are reviewed. The less-studied activities of NTF in non-neuronal systems (immune cells, germ cells, inflammatory processes, epithelial-mesenchymal interactions) are not discussed in this review (see Aloe *el al* on pages 7-14, and Mathison on pages 61-69 in this volume of *Biomedical Reviews).*

STRUCTURE OF THE HEUROTROPHIHS

• All NTF are small dimeric proteins with molecular weight of the monomers of 13 kD. As an average, all four mammalian NTF share about 50 % of amino acid sequence identity. Three-dimensional structure of NGF is described (22) and the molecule was shown to be quite unsimilar to other growth factors. It consists of seven B-strands in three antiparallel pairs with various hairpin loops at the both ends of the molecule. The core of the NGF monomer is maintained by three disulfide bridges and several hydrogen bonds. Two NGF monomers are associated by hydrophobia interactions. The structure of other NTF is not yet determined, but the amino acids establishing the basic structure are conserved among the NTF, whereas variable amino acids are mostly located in the hairpin loops, suggesting a similar basic structure for all NTF.

All NTF are synthesized as larger precursors (preproNTF) which are cleaved to mature forms by furin, an enzyme of subtilisin family of proteases (23), or a furin-like enzyme. Formation of the dimers occurs within the cell before secretion, the dimers are stably hold together and do not dissociate into monomers under normal conditions (24). Recently, BDNF/NT-3 heterodimers were obtained which had biological activities of both BDNF and NT-3, although 10-fold less potently than the corresponding homodimers (24).

RECEPTORS FOR THE MEUROTROPHIMS

• Two types of cell surface receptors for NGF were described by kinetic and chemical cross-linking studies (25-27). Type I receptors (slow receptors) bind NGF with high affinity ($Kd=10^{-u}$ M) and mediate the biological activity of NGF, whereas type II receptors (fast receptors) have low affinity for NGF ($Kd=10^{-9}$ M). Type II receptor (p75) was cloned in 1986 (28,29). In contrast, type I receptor resisted identification until 1991, when the normal counterpart of human colon carcinoma-derived trk (tyrosine kinase) oncogene was identified as a high-affinity NGF receptor (30-32). Two genes for highly homologous receptors were also cloned: trkB (33,34) and trkC (35), whereas trk is now often named as trkA.

The members of the trk family are protein tyrosine kinase receptors. The ligand specificities of the trk receptors (Fig. 1) were established mainly on NIH-3T3 fibroblast cells transfected with cDNA for a particular trk receptor, mostly by kinetic and receptor phosphorylation studies but also by stimulation of proliferation and malignant transformation of these cells upon NTF treatment. Thus, trkA is a high-affinity receptor for NGF (30,31), both BDNF and NT-4/5 bind trkB (33,36), and NT-3 is a primary ligand for trkC (35). In addition, NT-3 can also weakly bind to and activate trkA and trkB (37).

Trk receptors are glycoproteins of molecular weight of 140-145 kD with similar basic structure (Fig.2). The extracellular domains of trk receptors contain a leucine-rich motif in their N-terminal parts flanked by two cysteine clusters. All trks contain also two immunoglobulin-like domains in their extracellular part. Recently, the second immunoglobulin-like domain

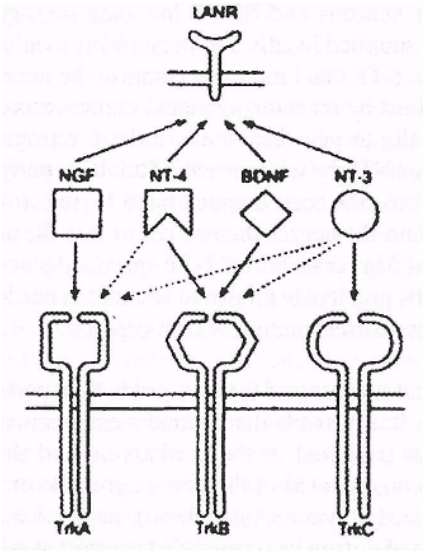

Figure 1. *Ligand-specificity of the neurotrophin receptors. NGF - nerve growth factor, BDNF - brain-derived neurotrophic factor, NT-3 and NT-4 - neurotrophin-3 and -4, LANR - low-affinity neurotrophin recepto, Trk - tyrosine kinase. The dashed arrows show weak interactions.*

was shown to be responsible for specific NTF recognition and high-affinity binding (38). The cytoplasmic portions of trk receptors contain tyrosine kinase domains which are highly homologous to each other (87-88 %), followed by short C-terminal tails.

In addition to full-length, catalytic trk receptors, there are also several receptor isoforms generated by alternative splicing. An isoform of trkA contains a six amino acid insert in the extracellular domain (39). Two truncated isoforms of trkB have been identified, which lack tyrosine kinase domain (34). Truncated trkB receptors are localized mostly in the non-neuronal cells, as astrocytes (40), Schwann cells (41), choroid plexus and ependyma (42). Even more isoforms are generated from the mRNA of trkC: four truncated forms, and three full-length forms with different inserts in the tyrosine kinase domain. The existence of multiple variants of trk receptors certainly adds to the complexity of the biological responses of NTF. For example, NT-3 induces DNA synthesis but not morphological transformation of NIH-3T3 fibroblasts transfected with trkC isoforms with the inserts, whereas both effects are seen when trkC of normal length is activated. Also, these isoforms do not mediate neurite outgrowth from rat pheochromocytoma PC 12 cells upon NT-3 treatment, which is again achieved by activating full-length trkC (43-45). However, the functional importance of the trk receptor isoforms *in vivo* are currently not known.

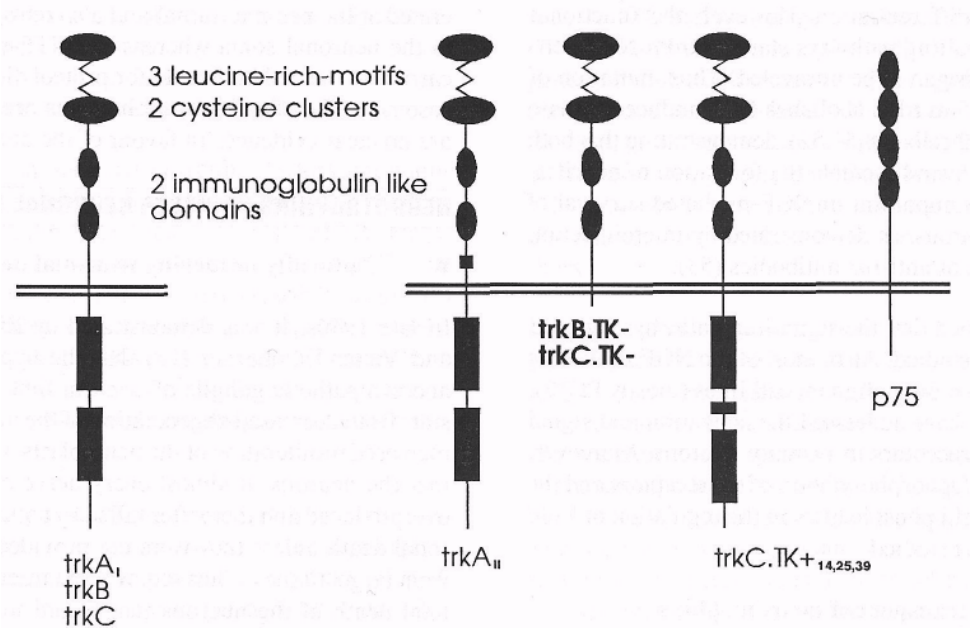

figure *2. Basic structure of the neurotrophin receptors. trkA $_n$ - trkA with a six amino acid insert absent in trkA $_p$ trkB. TK-andtrkC.TK-- truncated trkB and trkC, lacking the tyrosine kinase domain, trkC.TK+$_{1425j9}$ -full-length trkCvith 14,25, or 39 amino acids inserted in the tyrosine kinase domain, p75 - the low-affinity neurotrophin receptor.*

- **Activation of trk receptors**

Elucidation of the signalling pathways triggered by NTF binding to trk receptors is yet in its initial stage. Most studies have been carried out with rat pheochromocytoma PC 12 cells which express trkA and differentiate into sympathetic neuron-like cells upon NGF treatment. The signalling pathways of trkA appeared to be quite similar to those of other trk receptors. Binding of NGF leads to dimerization or oligomerization of the trkA receptors on the cell surface followed by reciprocal transphosphorylation of five of the tyrosine residues on both partners (46). These phosphotyrosine residues then act as docking sites for downstream proteins that trigger several intracellular pathways resulting in changes in the cytosolic proteins, cytoskeleton and the pattern of transcription factors. To date, four proteins are identified that bind to the phosphorylated tyrosine residues on NGF-activated trkA in PC12 cells. She adapter protein binds to phosphotyrosine residue (Y490) (47) followed by its phosphorylation that, through several intermediates, leads to the activation of *ras* protein. Phospholipase-yl binds to Y785 (47,48), phosphoinositol-3 (PI-3) kinase binds to Y751 (47), and Erk-1 (49), a serine-threonine kinase, is also shown to be associated with NGF-activated trkA. Obviously, more trkA-binding proteins will be identified in the future.

Each of the trkA-binding proteins activates specific downstream regulatory proteins (50), which ultimately leads to neuronal survival and differentiation. However, the functional meaning of the signalling pathways starting from NGF-activated trkA has just began to be unraveled. Thus, mutation of both Y490 and Y785 in trkA abolishes NGF-induced neurite outgrowth from PC 12 cells (48,51,52), demonstrating that both PCL-1- and *ras* pathways mediate the formation of neurites. *Ras* pathway is also important in NGF-mediated survival of primary sensory neurons, as demonstrated by microinjection of active *ras* protein or anti-ra,? antibodies (53).

It should be mentioned that the signalling pathways for trkB and trkC are poorly studied. Also, most of the NGF signalling studies are carried out on malignant cell lines (mostly PC12), whereas few studies have addressed the activation and signal transduction of trk receptors in primary neurons. Moreover, the mechanisms of dephosphorylation of trk receptors and the possible role of protein phosphatases in the regulation of NGF signalling are not yet studied.

- **Retrograde transport of neurotrophins**

The data about the NTF specificity and the activation of trk receptors have mostly been obtained in cultured cells where the whole cell surface is exposed to NTF. However, *in vivo* the NTF are picked up by the neurite terminals distant from the perikaryon, although in some cases, as NT-3 and BDNF for spinal motor neurons and BDNF for some sensory neurons, the NTF are supplied locally and presumably available also at the cell body. NTF that bind to receptors at the nerve terminal are internalized by receptor-mediated endocytosis and transported axonally to neuronal soma. Indeed, retrograde transport of all four NTF by wide variety of adult rat peripheral and central neurons has been demonstrated by injection of iodinated NTF into the nerves themselves or into the areas of innervation (54,55). Transported NTF specifically accumulated in the neurons previously known to respond to particular NTF and to express corresponding NTF receptors.

The biological meaning of the retrograde transport of NTF is still obscure. It is possible that second messenger pathways of NTF must be triggered in the perikaryon, and they are not efficient when generated in the nerve terminals or within the neurites. Indeed, it was recently shown that trkA was tyrosine phosphorylated during its retrograde transport as a NGF-trkA complex by rat sciatic nerve axons, therefore being able to trigger the signalling cascade after its internalization (56). In another experiment, part of the ^{125}I-NGF, retrogradely transported from neurite terminals of cultured neonatal rat sympathetic neurons has been stored intact in the perikarya for at least 15-18 hours (57). During that period, second messengers can be generated from the trkA tyrosine kinase domain exposed to the cytoplasmic side of the endocytotic vesicle. Alternatively, the appropriate second messenger(s) can be generated at the nerve terminal and also retrogradely transported to the neuronal soma whereas the NTF-receptor complex is carried to the cell body only for proteolytic degradation in lysosomes. Thus far, both mechanisms are possible and there are no clear evidences in favour of the one or another.

HEUROTROPHIMS PROMOTE NEURONAL SURVIVAL

- **Naturally occurring neuronal death**

In late 1940s, it was demonstrated by Rita Levi-Montalcini and Victor Hamburger (58) that the hyperplasia of sensory and sympathetic ganglia after extra limb transplantation results from decreased degeneration of the neurons and not from increased proliferation of the neuroblasts. Later it became clear that the neurons in almost every nerve center were initially overproduced and thereafter killed by naturally occurring neuronal death unless they were not provided by specific factors from target tissues. Thus removal of target tissue led to almost total death of the neurons that would innervate it, and implants of additional target resulted in enhanced survival of corresponding neurons. Target tissue can support only certain number of neurons innervating it (59,60), thereby regulating the density of its own innervation. This regulation occurs either via limiting amount of target-derived trophic factors that

a tissue can secrete or via limited access of the sites on the target where the factors are available.

Naturally occurring death takes place during certain stages of development which are specific for every neuronal population. For example, rat trigeminal ganglion neurons die between embryonic days 13-19 whereas the death period of rat sympathetic neurons is between postnatal days 3-7. When dissociated and grown *in vitro* soon after their birth, i. e. after the last mitosis, the neurons of DRG (61) and trigeminal ganglia (62) survive independently of any trophic factor. The program of death is initiated in the cultured trigeminal neurons at the time the first axons would have reached their tissue of innervation *in vivo* (63). Thus, the naturally occurring neuronal death is triggered in trigeminal neurons by a cell-autonomous genetic program and not by extrinsic factors at the time they first contact their target field. The molecular mechanism of this endogenous program and the developmental clock operating in the neurons are currently not understood.

The process by which cells are killed in the absence of trophic factors is called apoptosis. Apoptosis differs from necrosis, the death caused by unfavourable living conditions, in many ways. Most conspicuously, apoptosis is characterized by chromatin condensation, disruption of nuclear membrane, and specific, internucleosomal digestion of DNA. The mechanisms of apoptosis in many cell types, including neurons, are currently very intensively studied but not yet fully understood. In neurobiology, the term "trophic" actually means antiapoptotic, rather than nutritional.

- **Neurotrophins as antiapoptotic molecules**

NTF are clearly the target-derived trophic factors maintaining the innervating neurons and thereby regulating the density of target innervation. Also other factors as ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LfF), fibroblast growth factors (FGF), insulin-like growth factors (IGF) exert neurotrophic effect on some neuronal populations (64), but they are not discussed in this review. Thus NTF are only a part of the neurotrophic factors. In the following, evidences for NTF as *in vivo* target-derived neurotrophic factors are presented.

Studies of the developmental localization of NTF in the tissues correlated with the expression of their cognate receptors in the innervating neurons have given indirect proofs that all NTF can indeed be the endogenous target-derived trophic factors. Thus, in the developing epithelium of the branchial arches, which are innervated by trigeminal ganglion fibers during embryonic days 13-18 in rats, mRNA for all four NTF are expressed, the levels decreasing when the apoptosis period is over. At the same time, all three trk receptors are expressed in the trigeminal ganglia, and the trigeminal neurons respond functionally to all NTF in culture (65-67). Similarly, in the developing inner ear, mRNA for BDNF and NT-3 can be found in the sensory epithelia of vestibular apparatus and organ of Corti, respectively, at the time of their innervation by statoacoustic ganglion neurons. Correspondingly, mRNA for trkB and trkC can be found in the neurons of statoacoustic ganglion, and the neurons respond in culture to BDNF and NT-3 (68,69). The presence of NTF and their receptors at correct locations at the proper time is a necessary prerequisite for the target-derived neurotrophic activities.

Injection of function-blocking anti-NTF antibodies into developing embryos at the period of naturally occurring neuronal death must kill the sensitive neurons that are left without trophic support. Indeed, injection of NGF antiserum into neonatal rats almost completely destroyed the sympathetic ganglia and the tissues were consequently not innervated by sympathetic fibers (70). These effects were abolished by concomitant NGF injection. Applying function-blocking anti-NGF antibodies to mouse embryos in utero (71,72) resulted in specific elimination of trkA-expressing neurons in DRG. Injection of BDNF into the chorioallantoic membrane of quail embryos rescued substantial number of DRG neurons but also of nodose ganglion neurons, known to be BDNF-responsive and expressing trkB, from naturally occurring neuronal death (73). Similarly, monoclonal anti-NT-3 antibodies applied at the time of gangliogenesis, considerably reduced the number of neurons in DRG and nodose ganglia of developing quail embryos (74). The authors conclude that at the early stages of gangliogenesis the neuroblasts are not yet dependent from target for survival and the reduction in the number of neurons reflects the absence of proliferational signal normally provided by NT-3. However, the possibility that at later stages target-derived NT-3 also rescues part of sensory neurons from naturally occurring neuronal death *in vivo* was not excluded by the authors.

Strong evidences in support of the NTF as target-derived trophic factors *in vivo* have been obtained from the transgenic mice with disrupted genes for NTF and their receptors (75,76). In these mice, substantial losses of those peripheral neurons that are known to be sensitive to a particular NTF are reported. Moreover, the losses in peripheral neurons of the NTF-deficient mice were qualitatively comparable to the losses in the mice deficient in their cognate trk receptors (77). The absence of appropriate neurons in the NTF-deficient mice is most easily explained by their apoptotic death in the absence of necessary NTF. The death of the neurons lacking trk receptors reveals that their program to die in the absence of certain NTF is effective even if the receptor for that factor is not expressed.

- **Models of the trophic effect**

The mechanisms by which NTF neutralize the apoptotic process are not fully understood, but are currently very intensively studied. In the following, several models are briefly described, but none of them is yet sufficient to explain the antiapoptotic effects of NTF. These mechanisms may, however, well be intertwined or act independently of each other. Certainly in the near future new data will complete the picture.

Overexpression of protooncogene *bcl-2* which has been shown to be an antiapoptotic molecule in hematopoietic cells, increased survival of mouse sympathetic neurons but also of embryonic chick cranial and autonomic neurons after NGF deprivation 777 *vitro* (78,79), whereas blocking of endogenous *bcl-2* by antisense RNA killed BDNF-dependent chick sensory neurons in the presence of BDNF (80). *Bcl-2* is expressed in the nervous system of developing mouse (81). The DRG neurons and motor neurons from transgenic mice overexpressing *bcl-2* gene in the nerve cells showed enhanced survival in the absence of NGF *in vivo* and *in vitro* (82). However, not all neurons were protected by *bcl-2* in these experiments. Also, the nervous system appeared to be normal in /3c/-2-deficient transgenic mice (83) suggesting that *bcl-2* is not the only molecule mediating survival of the neurons. Interestingly, there are yet no data about the possible regulation of *bcl-2* level or activity by NTF.

By another model, NTF rescue neurons from apoptotic death by counteracting the oxidative stress triggered in their absence. In favor of that, NGF-deprived sympathetic neurons have been rescued from apoptotic death by the antioxidant compound N-acetyl-L-cysteine (84). However, direct effect of NTF on the level of the antioxidant molecules, such as glutathione, in rescued neurons has not been demonstrated.

Inhibition of RNA or protein synthesis has been shown to block the apoptotic death of sympathetic neurons deprived of NGF, suggesting that *de novo* synthesis of killer proteins is necessary for apoptosis to occur, and that NTF normally neutralize their synthesis (85). In favor of this, aurintricarboxylic acid as an inhibitor of endonucleases, the putative killer proteins, inhibited DNA fragmentation and promoted survival of NGF-deprived sympathetic neurons from apoptotic death (86). Alternatively, inhibition of RNA and protein synthesis also upregulates the level of glutathione in apoptotic neurons, thus counterbalancing the oxidative stress (87). Again, similar upregulation of gluthatione by NTF remains to be demonstrated. Specific upregulation of *c-jun* mRN A in NGF-deprived apoptotic sympathetic neurons, as well as blocking of apoptosis in these neurons by *anli-jun* antibodies suggests that transcription of the genes for putative killer proteins is activated by *c-jun,* and that NGF neutralizes their transcription (88). But upregulation of *c-jun* can also reflect the abortive attempt of postmitotic neurons to enter the cell cycle after NGF deprivation, which ultimately results in apoptotic death.

- **The effect of** NTF **on the motor neurons**

The trophic activities of NTF reviewed above are mostly studied on peripheral sympathetic and sensory neurons. Similar functions of the NTF on the spinal cord motor neurons *in vivo* are not yet unequivocally demonstrated. On the one hand, BDNF, NT-3 and NT-4, but not NGF, clearly affect motor neurons in experimental situations by increasing the activity of choline acetyltransferase *in vitro* (89), and by rescuing lesioned motor neurons trophically when injected into embryonic chick muscles (90,91). Motor neurons express trkB and trkC during embryogenesis, the skeletal muscles they innervate express BDNF, NT-3 and NT-4, and motor neurons can transport these factors retrogradely (41,55). On the other hand, transgenic mice with null mutations in the BDNF or NT-3 gene, but also in the genes of trkB or trkC, have a normal number of motor neurons in their spinal cords (75). Also, injection of function-blocking anti-NT-3 antibodies into embryonic chick sciatic nerve has no effect on the motor neurons whereas it leads to the death of NT-3-dependent neurons in the DRG (92). Thus, although BDNF, NT-3 and NT-4 can potentially rescue the motor neurons from naturally occurring neuronal death, they are not the only factors that can do this. For example, CNTF and LIF, the trophic factors not related to NTF, also have trophic effect on motor neurons (64). It is possible that the trophic potency of NTF for the motor neurons is used in traumatic or pathological situations, because BDNF is also expressed in the glial cells at the distal stump of the damaged nerve (41) and BDNF, NT-3 and NT-4 are expressed within the spinal cord itself (41). The factor(s) actually regulating the number of motor neurons in embryogenesis have not yet been identified with certainty.

MEUROTROPHIMS IN THE AXOHAL GUIDANCE

NGF was named so by its virtue to induce neurite outgrowth from the peripheral ganglia and to direct their growth. Thus, microinjecion of NGF into the brain ventricle of neonatal rats evoked massive ingrowth of sympathetic fibers into brain tissue towards the source of NGF, where they normally never grow (93). These results lead to the hypothesis that during embryogenesis, NGF, secreted from the preinnervational tissues, form a gradient in the embryo which is recognized by the growing axons who can then navigate toward the correct target. This theory found more support from the experiments where local application of NGF from micropipette to the growth cones of cultured embryonic DRG explants led the growth cone to turn toward the pipette and even to follow its movement (94). Also, experiments where only the growth cones but not

cell bodies of cultured sympathetic neurons were exposed to NGF demonstrated that NGF can promote neurite growth locally at the growth cone (95). However, the neurites studied in the abovementioned experiments were not the naive pioneers but rather regenerating neurites, as they were studied at the stages when neuritic contacts had already been established. Although primary neurite growth is similar to regenerational growth, these two are not necessarily identical. Thus far, there are no solid data about the neurotropic role of the NTF for primary neurites. The word "tropic" is derived from Greek *"trope"'* which means direction, attraction (see Lakke on pages 95-102 in this volume of *Biomedical Reviews).*

NTF secreted from the embryonic tissues can, however, direct the terminal branching of the axons and formation of the "fine" axon-to-cell contacts. This hypothesis was recently substantiated by the finding that some NTF may also retract certain growth cones of early sensory ganglion neurons instead of attracting them (96). In these experiments, neurites were induced from preinnervational trigeminal ganglionic explants by one NTF. Another NTF was then added, and its effect on the growth cones was assessed. It appeared that BDNF and NT-4 caused rapid collapse and retraction of the growth cones induced by NGF or NT-3, but in other combinations, the second NTF caused further advancement of the growth cones. The same phenomena were also described for regenerating trigeminal ganglion and DRG neurites. All four NTF are expressed in the cutaneous target of the trigeminal neurons at the time when their axons contact it (67), and the attraction-retraction mechanism would be useful to direct every axonal branch to its innervational partner through the field of many factors.

An abnormally dense local sympathetic innervation of the pancreatic islets was described in transgenic mice overexpressing NGF in this tissue, which was explained by the increase in terminal branching of the sympathetic fibers by excess NGF (97).

In transgenic mice deficient in NTF or their receptors, severe reduction of respective sensory or sympathetic innervation has been reported resulting from the death of corresponding neurons (75). However, few conclusions can be drawn from these studies about the possible neurite-guiding role of NTF, as the correctness of axonal pathways of these neurons at the earlier stages, i.e. before the commencement of programmed neuronal death was generally not analyzed. It was reported, however, that some vestibular afferents were grown into the connective tissue adjacent to the vestibular apparatus in BDNF-deficient mice but the nerve endings had not contacted it (98). The vestibular apparatus expressed BDNF mRNA when axons of statoacoustic ganglion were growing towards it, and thereafter (68,69). The absence of wrong projections to it suggests that BDNF does not determine the course of the vestibular nerve. Rather, it determines the terminal branching and the extent of local innervation within the tissue. However, it is also possible that the few survived vestibular neurons are not BDNF-dependent and their axons are directed by some other factor.

The neurite-growth promoting activities of NTF can be used in reparative regeneration of the axons. Indeed, it has been demonstrated that the crush of adult rat sciatic nerve is followed by upregulation of NGF, BDNF and NT-4 transcripts in the distal part of the crushed nerve (41,99), most probably in the distal Schwann cells that have lost the contact with the axons. Also, mRNA for truncated trkB and trkC, but also for p75, were upregulated in the Schwann cells. By current model, the NTF secreted by Schwann cells bind receptors on the surface of the same cells and are thus presented for the axonal stumps to facilitate their regeneration.

Several experimental data indicate that promoting the neuronal survival and neurite outgrowth are two different activities of the NTF which occur independently of each other. 6-thioguanine, an inhibitor of a NGF-specific kinase, interferes with neurite growth in sympathetic and sensory neurons without affecting their NGF-dependent survival (100). Also, aurintricarboxylic acid, an inhibitor of endonucleases (86), as well as several antioxidant substances (84) prevented apoptotic death of NGF-deprived neuronal cells but did not induce or maintain neurites in these cells. A chimeric NGF/BDNF protein induced neurite outgrowth from cultured nodose ganglia but had no effect on the survival of nodose neurons (101). As the chimeric NTF activated trkB at similar levels as did the wild-type BDNF, it was suggested that qualitative differences (perhaps phosphorylation of different tyrosine residues of trkB receptors) are responsible for neurite-promoting and survival-promoting activities of NTF. Also, an isoform of trkC with an insert between amino acids 711 and 712 has specifically lost its ability to induce neurites in response to NT-3 suggesting that the signalling pathway for neurite outgrowth lies in this region. Further, NGF-induced neurite outgrowth from PC12-derived cells was inhibited by mutating both Y490 and Y785 in trkA (51). These results suggest that the signalling pathways of the NTF-mediating neurite outgrowth and survival of the neuronal cells may be mediated by different intracellular pathways initiated from separate regions of trk-receptors. This hypothesis is still lacking definitive proof.

Thus, although NTF clearly have a neurite growth-promoting activity, its biological meaning *in vivo* has not deserved sufficient attention and is mostly not understood.

LOW-AFFINITY NEUROTROPH1N RECEPTOR

The low-affinity NTF receptor p75 belongs structurally to the

family of tumor necrosis factor receptors (TNFR), which includes also Fas, CD27, CD30 and CD40. p75 binds all NTF with similar equilibrium kinetics with Kd of 10^{9} M, but the dissociation constants are different for different NTF (102).

The existence of p75 has been known since 1986, but its functional significance has been and still is the matter of debate and has become somehow understood only recently. It has been argued that p75 receptor is not necessary for NTF signalling, as trk receptors are activated in the fibroblasts having no p75. Moreover, biological effects of NGF were not hampered when its binding site for p75 but not for trkA was mutated (103), or when binding of NGF to p75 was blocked with specific antibodies (104). Also, fibroblasts cotransfected with p75 and trk receptors did not display an increase in the high-affinity sites. So, the existence of two-receptor system for NTF was severely questioned. However, p75 clearly does participate in the activities of NTF, as will be exemplified below by several recent results.

First, there are new data suggesting that p75 may still be necessary for the formation of the high-affinity NTF receptor complex, as most of trk receptors alone bind NTF with low affinity. Indeed, only 1-2 % of the NGF binding sites on trkA-transfected fibroblasts were of high-affinity, whereas the remaining bound NGF with low affinity. Moreover, more high-affinity NGF binding sites were generated when neurons were cotransfected with trkA and p75 with the ratio of p75/trkA being 1/10 (105). This p75/trkA ratio has been reported for sympathetic neurons, whereas in cotransfected fibroblasts it has been much smaller. These data suggest that interaction of p75 with trk-receptors may be necessary to create high-affinity binding of NTF to trks. However, heterodimerization of these two receptors has never been demonstrated in spite of several attempts (27), so the biochemical nature of this putative interaction cannot be explained today.

Several recent works have also demonstrated that the presence of p75 has a modulatory effect on the activation of trk receptors. Occupancy of p75 on the PC12 cells by BDNF led to reduction in the formation of NGF-trkA complexes, in phosphorylation of trkA by NGF, and in the level of NGF-induced *c-fos* mRNA (106). Transfection of trkA-expressing sympathoadrenal progenitors with p75 resulted in increase of NGF-induced trkA phosphorylation, and the effect was not obtained with mutant NGF not binding p75 (107). These results demonstrate that p75 may seriously affect functional effects of NTF on the neurons. This idea obtained recently an important support when receptor-mediated retrograde transport of iodinated NT-4 and BDNF, but not of NGF or NT-3, by peripheral axons of embryonic rat sensory and sympathetic neurons was specifically inhibited by function-blocking anti-p75 antibodies or by recombinant extracellular domain of p75 (55). In addition, the ability of NT-4 but not of other NTF to promote neurite outgrowth or survival of neuronal cells was significantly reduced when its binding site for p75 was mutated (108). Thus, p75 can discriminate the NTF in retrograde transport and biological function.

A direct apoptosis-triggering role for unoccupied p75 has been demonstrated in a cerebellar cell line (109). This is not surprising as TNFR and Fas antigen have also been shown to lead the cells to apoptosis. In addition, blocking of unoccupied p75 by antisense oligonucleotides resulted in apoptotic death of embryonic mouse sensory neurons (110). Interestingly, the same oligonucleotides increased the survival of newborn mouse sensory neurons demonstrating that p75 can act in an opposite manner at different stages of development. The biochemical mechanism of the apoptotic effect of p75 is not understood, and no putative killing proteins binding to its cytoplasmic domain have been described. Binding of NGF to trkA clearly neutralizes the apoptosis in neurons. Whether p75 is counteracting this process *in vivo,* is an intriguing possibility.

The transgenic p75-deficient mice lost a substantial population of sensory neurons (111,112), and reduction in the NGF-sensitivity of trigeminal neurons was reported (113). These results once more prove the functional importance of p75 for neuronal cells. Importantly, absence of sympathetic innervation of pineal gland and some sweat glands in the footpad, but not of other organs, was also observed in p75-deficient mice (112), demonstrating that p75 is also important in the establishment of innervation of some organs.

Thus, p75 seems to participate in every aspect of NTF action. Its role is, however, a complex one which hampers the understanding. Recent developments fitness a reappraisal of previously antagonistic views about the role of p75 in the ontogenesis of the nervous system.

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