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Therapeutic Potential of Neurotrophic Factors in Neurodegenerative Diseases

Yossef S. Levy, *Yossi Gilgun-Sherki*, *Eldad Melamed* and *Daniel Offen*

Laboratory of Neurosciences, Felsenstein Medical Research Center, and Department of Neurology, Rabin Medical Center, The Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

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Abstract

There is a vast amount of evidence indicating that neurotrophic factors play a major role in the development, maintenance, and survival of neurons and neuron-supporting cells such as glia and oligodendrocytes. In addition, it is well known that alterations in levels of neurotrophic factors or their receptors can lead to neuronal death and contribute to the pathogenesis of neurodegenerative diseases such as Parkinson disease, Alzheimer disease, Huntington disease, amyotrophic lateral sclerosis, and also aging. Although various treatments alleviate the symptoms of neurodegenerative diseases, none of them prevent or halt the neurodegenerative process. The high potency of neurotrophic factors, as shown by many experimental studies, makes them a rational candidate co-therapeutic agent in neurodegenerative disease. However, in practice, their clinical use is limited because of difficulties in protein delivery and pharmacokinetics in the central nervous system. To overcome these disadvantages and to facilitate the development of drugs with improved pharmacotherapeutic profiles, research is underway on neurotrophic factors and their receptors, and the molecular mechanisms by which they work, together with the development of new technologies for their delivery into the brain.

Neurotrophic factors (NTFs) are diffusible peptides secreted from neurons and neuron-supporting cells. They serve as growth factors for the development, maintenance, repair, and survival of specific neuronal populations, and they act via retrograde signaling from target neurons by paracrine and autocrine mechanisms.[1]

During development, NTFs promote neuronal survival, stimulate axonal growth, and play a key role in the construction of the normal synaptic network.[1,2] In adulthood, they help to maintain neuronal functions and specific neuronal phenotypes.[3] There is emerging evidence that the different families of NTFs work in synchronization and influence each other.[4] Therefore, any alterations in their local synthesis, transport and/or signaling (e.g. binding, internalization, receptor synthesis, etc.) due to local damage, aging, mutations, or polymorphisms could adversely affect neuronal survival and lead to neuronal death.[5-9] In addition, a large body of research suggests that some NTFs modulate neuronal plasticity during aging and pathologic states such as trauma or neurodegeneration.[3] Indeed, studies show that loss of endogenous target-derived trophic support for selective neuronal populations might contribute to the pathology of neurodegenerative diseases, such as Parkinson disease (PD), Alzheimer disease (AD), Huntington disease (HD), and amyotrophic lateral sclerosis (ALS).[6] Recently, researchers have directed their attention to the identification of those conditions promoting human neuronal survival and repair in neurodegenerative diseases and developing neuronal grafts to replace injured or diseased brain.[10] Studies on implanted cells and tissue suggest that pretreatment with NTFs might improve cell survival.[11-13] To summarize the current data on NTFs, we searched the Medline database (PubMed) from January 1954 to January 2004. The key words used were the names of NTFs or 'NTFs' together with the names of neurodegenerative diseases. The search was augmented by using references cited in these published articles.

This review summarizes the current data on NTFs and stresses their important contribution to neuronal maintenance and survival and their therapeutic potential in neurodegenerative diseases.

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1. Families of Neurotrophic Factors (NTFs)

NTFs belong to several superfamilies of structurally and functionally related molecules, as follows: (i) nerve growth factor (NGF)-superfamily; (ii) transforming growth factor (TGF)-β superfamily, consisting of the glial-cell-line-derived neurotrophic factor (GDNF) family, the TGFβ family, and the bone morphogenetic protein (BMP) family; (iii) neurokine or neuropoietin superfamily; and (iv) non-neuronal growth factors (see figure 1).

1.1 Nerve Growth Factor (NGF) Superfamily

The mammalian NGF superfamily, originally called neurotrophins, includes NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NTF3), and neurotrophin-4/5 (NTF5)[14] [figure 1]. Two additional neurotrophins, neurotrophin-6 (NTF6)[15] and neurotrophin-7 (NTF7),[16] have been identified only in fish.

1.1.1 Identification and Role

NGF was the first neurotrophin identified. The human NGF gene is located on the short arm of chromosome 1 (1p22)[17] and codes for a polypeptide of 118 amino acids, after cleavage. It exists naturally as a noncovalently bound homodimer.

The gene for BDNF was cloned by Leibrock et al.[18] and is located on human chromosome 11:11p13.[19] It has close homology to NGF, with 51 identical amino acids.

In the contiguous regions between NGF and BDNF, NTF3 was present as observed using PCR. It was cloned by Ernfors et al.[20] and Hohn et al.[21] and is located on human chromosome 12 (12p13).[19]

Neurotrophin-4 was found after a long period of investigation and cloned finally in Xenopus by Hallbook et al.[22] The equivalent human complementary DNA (cDNA) that was sufficiently different from Xenopus neurotrophin-4 to be considered a separate gene was called neurotrophin-5.[23] Thereafter, researchers realized that the two are homologous genes, and this neurotrophin, which is present on human chromosome 19 (19q13.3), is now termed NTF5.[24]

1.1.2 Neurotrophin Receptors

The neurotrophins act by attaching to three high-affinity neurotrophic tyrosine receptor kinases (Trk/NTRK)[25] and to the single-affinity p75[26] (table I). TrkA (NTRK1) is the preferred receptor for NGF, NTF6, and NTF7, and TrkB (NTRK2) is the preferred receptor for BDNF and NTF5.[16,27,28] NTF3 is the only neurotrophin capable of activating TrkC (NTRK3), its preferred receptor, but it also acts as a secondary ligand for both TrkA and TrkB.[29,30] The p75 neurotrophin receptor (p75NTR; NGFR) binds all of the neurotrophins equally, with relatively single-affinity compared with Trk binding[30] (table I). When Trk receptors and p75NTR are coexpressed, the latter enhances the ability of Trk receptors to bind and respond to neurotrophins and sharpens the discrimination of Trks for their preferred neurotrophin ligands. Peripheral tissues produce low concentrations of neurotrophins to maintain appropriate levels of neuronal survival and innervation, and p75NTR appears to act as a coreceptor that allows the Trks to respond to limiting neurotrophin levels. Indeed, the loss of sympathetic and sensory neurons and the progressive peripheral neuropathy observed in adult p75NTR null mice[31,32] likely reflect reduced Trk activation in peripheral neurons.

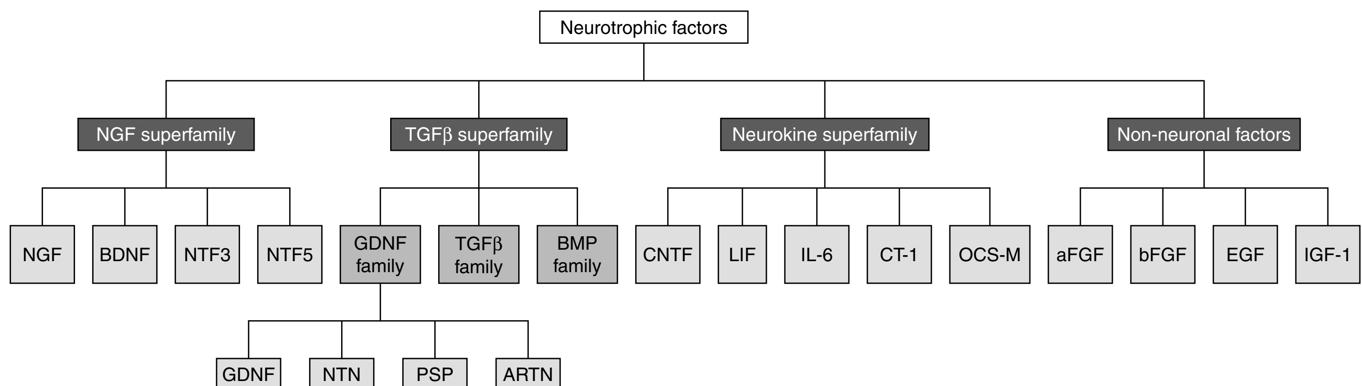

Fig. 1. Classification of the neurotrophic factor families in humans. **aFGF** = acidic fibroblast growth factor; **ARTN** = artenin; **BDNF** = brain-derived neurotrophic factors; **bFGF** = basic fibroblast growth factor; **BMP** = bone morphogenic protein; **CNTF** = cilliary neurotrophic factor-1; **CT-1** = cardiotrophin-1; **EGF** = epidermal growth factor; **GDNF** = glial cell line-derived neurotrophic factor; **IGF-1** = insulin-like growth factor; **IL-6** = interleukin-6; **LIF** = leukemia inhibitory factor; **NGF** = nerve growth factor; **NTF** = neurotrophin; **NTN** = neurturin; **OCS-M** = oncostatin-M; **PSP** = persephin; **TGFβ** = transforming growth factor-β.

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Table I. The receptors of neurotrophic factors

Neurotrophic factor	Receptor	Comments
Neurotrophins superfamily		
Nerve growth factor	TrkA/p75	Trk-high affinity, p75-low affinity
Brain-derived neurotrophic factor	TrkB/p75	Trk-high affinity, p75-low affinity
Neurotrophin-3	TrkC, B, A/p75	Trk-high affinity, p75-low affinity
Neurotrophin-4/5	TrkB/p75	Trk-high affinity, p75-low affinity
Transforming growth factor-β superfamily		
Glial cell line-derived neurotrophic factor	GFRα1	Activation through RET
Neurturin	GFRα2	Activation through RET
Persephin	GFRα3	Activation through RET
Artenin	GFRα4	Activation through RET
Neurokines superfamily		
Cilliary neurotrophic factor	CNTFR	One subunit of LIFRβ and one subunit of gp130
Leukemia inhibitory factor	LIFR	One subunit of LIFRβ and one e subunit of gp130
Interleukin-6	IL-6R	Two subunit of gp130
Cardiotrophin-1	CTF-1R	One subunit of LIFRβ and one subunit of gp130

CNTFR = cilliary neurotrophic factor receptor; **CTF-1R** = cardiotrophin-1 receptor; **GDNF** = glial cell line derived neurotrophic factor; **GFR** = GDNF-family receptor; **gp** = membrane glycoprotein; **IL-6R** = interleukin-6 receptor; **LIFR** = leukemia inhibitory factor receptor; **p75** = neurotrophin receptor/nerve growth factor receptor; **RET** = proto-oncogene receptor tyrosine kinase; **Trk** = tyrosine receptor kinase (also known as NTRK1–3).

Over the last decade, numerous studies have shown that p75NTR can act as an apoptotic receptor during development and following injury, but there has been controversy about the precise ligand requirements for these effects.[33] Mature neurotrophins are not effective activators of p75NTR-induced apoptosis, and high nonphysiologic concentrations are often required to induce even modest levels of cell death. Neurotrophins are synthesized as precursors that can be cleaved by furin and proconvertases to produce mature NGF. Using a furin-resistant form of proNGF, Hempstead and colleagues[34,35] found that proNGF binds p75NTR with high affinity and is a potent inducer of p75NTR-dependent apoptosis, in sympathetic neurons and oligodendrocytes. These investigators also showed that proNGF does not bind TrkA and suggested that proNGF is an apoptotic ligand that is specific for p75NTR. Moreover, the recent identification of sortilin as a p75NTR coreceptor necessary for proNGF-induced cell death is a major breakthrough.[35] Sortilin is a type I transmembrane protein expressed in a wide variety of tissues but is most abundant in the CNS during development and in adults. Nykjaer et al.[35] recently discovered that the NGF prodomain directly binds the extracellular domain of sortilin and, through crosslinking studies, established that p75NTR and sortilin form a receptor complex that binds proNGF at the cell surface. Both receptors appear to be required to transduce the apoptotic effects of proNGF. Blocking the interaction of proNGF with sortilin inhibits proNGF-mediated apoptosis, whereas expression of exogenous sortilin in Schwann cells, which normally express only p75NTR, renders these cells sensitive to the apoptotic effect of proNGF.[35]

In addition to its roles as a Trk coreceptor and regulator of apoptosis, p75NTR has recently emerged as a key player in the regulation of neuronal growth. Definitive data linking p75NTR to this function came from studies of Yamashita and colleagues[36] who demonstrated that myelin-associated glycoprotein-induced growth inhibition and RhoA activation were attenuated in sensory and cerebellar granule neurons derived from p75NTR null mice. These findings indicate that p75NTR-Nogo receptor complex is required for myelin-based growth inhibitor signaling. Furthermore, Mi et al.[37] found that the extracellular domain of LINGO-1 (LRR and Ig domain-containing, Nogo receptor-interacting protein) binds both Nogo receptor (reticulon 4 receptor, RTN4R) and p75NTR and showed that full-length LINGO-1 can be immunoprecipitated with either RTN4R or p75NTR.

Immunoreactivity for the p75NTR was found in numerous cells of the paraventricular and supraoptic nuclei of the hypothalamus, pituitary stalk, and median eminence, and immunoreactive TrkA and TrkB receptors were found in normal and neoplastic endocrine cells in human pituitary adenomas.[38] These findings suggest that

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neurotrophins play a key role in the hypothalamic-hypophyseal endocrine system.[39]

Immunohistochemical and *in situ* analysis of the distribution of p75NTR in rat and primate brain[40-44] and colocalization of choline acetyltransferase (ChAT) and p75NTR immunoreactivity[41-43] show that p75NTR is almost exclusively located on basal forebrain cholinergic neurons. More recently, examination of the distribution of TrkA protein and mRNA in rat and human brain shows that TrkA is also expressed on basal forebrain cholinergic neurons,[45,46] although there are some TrkA-positive, p75NTR-negative neurones in the striatum.[40-42,46] By contrast, TrkB and TrkC are much more widely distributed.[47]

In post-mortem human brain studies, moderate immunostaining for TrkA, TrkB, and TrkC was identified in neurons, and weak immunostaining in astrocytes; strong Trk immunostaining was found in reactive astrocytes, such as glial scars, glial fibrillary acidic protein (GFAP) and various diseases and injuries. Indeed, Trk staining can be used to distinguish astrocytes from oligodendrocytes. The synthesis of the neurotrophins and receptors in astrocytes indicates that they participate in autocrine and paracrine functions.[38,48]

1.1.3 CNS Distribution

Neurotrophins are produced and released by target cells, taken up by nerve terminals in the brain, and retrogradely transported to neuronal cell bodies where they exert their trophic effects. They may be found in various neuronal subsets and glial cells in the trigeminal ganglion at several ages of gestation and through adulthood.[49] Treating mouse embryos with anti-NGF antibodies reduces the number of trigeminal sympathetic and dorsal root ganglion neurons to 20% of their control numbers.[50] Moreover, most are localized mainly in the hippocampus, except for BDNF, which is primarily localized in the third and fifth pyramidal layers of the cerebral cortex, hippocampus, caudate nucleus, putamen,[51] and striatum[52] and in ventral tegmental area (VTA), the cell body region of mesolimbic dopamine neurons.[53]

Immunocytochemical studies of human brain, have shown that BDNF is primarily localized in the third and fifth pyramidal layers of the human cerebral cortex. Immunostaining reveals neurons and surrounding neuropil. The distribution is more abundant in the insular and temporal cortices than in the primary motor and sensory cortices. Scattered pyramidal neurons show intense labeling in the cells of deeper pyramidal layers.[51] Within the hippocampus, the pyramidal cell layer of the hippocampal subfields CA1 through CA4 and the granule cell layer of the dentate gyrus exhibit positive immunostaining. Intense immunostaining was also observed in the neuropil of the pyramidal layers of CA3–CA4 subfields and the hilar region.[51] All amygdaloid nuclei show BDNF-immunoreactive neurons and fibers. The lateral nucleus shows abundant neurons with intense perikaryal staining and a faintly labeled neuropil.[51] Murer et al.[51] report that both the caudate nucleus and putamen exhibit non-homogenous immunolabeling of BDNF, but the distribution in the substantia nigra pars compacta (SNpc) is not described. Direct comparison of the staining patterns on BDNF- and acetylcholinesterase-labeled sections show that acetylcholinesterase-deprived regions are intensely stained with BDNF. BDNF-immunostained regions are histologically identified as striosomes. At the cellular level, the staining is restricted to neuronal processes, while the cell bodies are very faintly stained. In the mesencephalon, the regions corresponding to the dopaminergic cell groups exhibit fine punctate cytoplasmic staining of the proximal dendritic branches.

In another study of human brain,[52] immunoreactive BDNF is found distributed in neurites of striatum. TrkB, the receptor for BDNF, is also found in neuronal perikarya in neocortex and striatum and in reactive astrocytes. The presence of both receptor and factor in the neocortical perikarya suggests an autocrine function in those neurons while the presence of factor in neurites of striatum suggests that the factor is not synthesized locally in the striatum but is in the process of being taken up by retrograde transport.

Data from human SNpc show strong immunostaining for both BDNF and NGF in the perikarya of melanin-containing neurons and in proximal dendritic branches in SNpc. The surrounding neuropil, possibly consisting of neuronal and astroglial processes, also displays immunoreactivity for both BDNF and NGF. The melanin-containing neurons of SNpc are intensely immunostained with anti-NTF3 antibody.[54-56]

NTF3 levels have been determined in various portions of human brain[57] while localization of NGF, BDNF, NTF3, and NTF5 has been demonstrated in various neuronal subsets and glial cells in human trigeminal ganglion from gestation through to adulthood.[49] In addition, the melanin-containing neurons were intensely immunostained with anti-NTF3 antibody (for a summary of neurotrophin distribution,[58] see table II). The results of these studies have been corroborated by gene knockout experiments, wherein the deletion of particular neurotrophins causes the loss of only particular subsets of neurons.[59,60] In the human hippocampus, immunoreactive labeling for NGF, BDNF, NTF3, and NTF5 is found in neurons and processes at all ages tested (from 23 weeks gestation to 68 years). Staining is generally higher at earlier ages except for BDNF immunostaining in nerve fibers, which extended

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Table II. Neurotrophin targets at the central nervous system

Neurotrophins	Central nervous system targets
Nerve growth factor	Basal forebrain cholinergic neurons, striatal cholinergic neurons, Purkinje cells
BDNF	Spinal motor neurons, basal forebrain cholinergic neurons, substantia nigra dopaminergic neurons, facial motor neurons, retinal ganglion cells
NTF3	Basal forebrain cholinergic neurons, locus ceruleus neurons
NTF5	Basal forebrain cholinergic neurons, locus ceruleus neurons, motor neurons, retinal ganglion cells

BDNF = brain-derived neurotrophic factor; **NTF3** = neurotrophin 3; **NTF5** = neurotrophin 4/5.

in distribution with age, thus suggesting that neurotrophins are functional in the hippocampus throughout life.[61]

1.2 Transforming Growth Factor (TGF)-β Superfamily

The TGFβ superfamily, consisting of more than 30 related members in mammals, including TGFβs, BMPs, activins, growth and differentiation factor (GDF), and Nodal, fulfills key functions during development and in maintaining tissue homeostasis.[62,63] The superfamily is divided into two general branches: BMPs/GDF and TGFβ/Activin/Nodal, whose members have diverse, albeit often complementary, effects.[62]

1.2.1 TGFβ Family

The TGFβ family consists of three members in mammals: TGFβ1, TGFβ2, and TGFβ3. TGFβ1 is expressed principally by glial cells and is not present in significant amounts in the mature nervous system. TGFβ1 is thought to function principally following injury to the nervous system, where its expression is dramatically induced in microglial cells. In some neurons, TGFβ1 is a component of the response to neurodegeneration or trauma and its synthesis and secretion are elevated in these settings.[58]

TGFβ2 and TGFβ3 are widely expressed in both the peripheral nerve system and the CNS. Early in development, TGFβ2 is associated with developing fiber tracts, and later in development it is found in astrocytes. TGFβ3 acts as a mitogen for amacrine cells in the developing retina. In the adult, TGFβ2 and TGFβ3 are found in many neuronal populations and in both astrocytes and Schwann cells.[58]

There is evidence that TGFβ2 and TGFβ3 act as morphogenic and differentiation factors for dopaminergic neurons in the striatum and midbrain.[64] It appears that during development, TGFβ2, TGFβ3, and GDNF are expressed locally in regions in which these neurons reside, while at later times, these factors are expressed principally within the projection fields of these neurons. These findings have been interpreted to support the view that members of the TGFβ family act as potent survival factors for midbrain dopaminergic neurons.

1.2.2 Bone Morphogenetic Protein Family

BMPs were first identified on the basis of their effect on bone formation. Relatively little is known about their range of actions in the nervous system. Nevertheless, it is clear that these factors have important roles early in the development of the nervous system. BMP2 influences the differentiation of multipotential cells of the neural crest into a neuronal phenotype. The BMPs also have significant roles as trophic factors. BMP2 and BMP6 act as survival factors for neurons of the cerebral cortex and cerebellum. BMP6 is widely expressed in the developing and mature nervous system, and there is growing evidence suggesting that BMP family members act in concert with other factors to regulate neuronal differentiation and survival.[58]

1.2.3 Glial-Cell-Line-Derived Neurotrophic Factor (GDNF) Family

The GDNF family is distantly related to the TGFβ superfamily and includes GDNF[65] and three structurally related members called neurturin (NTN),[4] persephin (PSP),[66] and artemin (ARTN)[67] [figure 1].

Identification and Role

GDNF, the first-studied member of this family, is a potent NTF. Initial studies indicated that GDNF supports the survival of dopaminergic neurons of the SNpc *in vitro* and *in vivo*. Since then, many studies with *in vitro* and *in vivo* models have pointed to a role of GDNF in the neuritic outgrowth or survival of mesencephalic dopaminergic neurons, cranial and spinal cord motor neurons, brainstem noradrenergic neurons, basal forebrain cholinergic neurons, Purkinje cells, and certain groups of dorsal ganglia and sympathetic neurons.[6,68] The human GDNF gene uses a promoter distinct from that of the rodent GDNF gene. The human promoter contains sites for binding a number of transcription factors responsive to environmental signals, which may account for the complex expression patterns of GDNF in neurons and glia.[69]

NTN has been identified and cloned on the basis of its survival-promoting effects on sympathetic neurons and on sensory neurons of the nodose and dorsal root ganglia (DRG). It is expressed in the developing and adult nigrostriatal systems and promotes survival

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of embryonic dopaminergic neurons *in vitro* and striatal neurons *in vivo*.[70]

PSP is a protein with a 40% similarity to GDNF and NTN, and its mRNA has been found in all tissues examined in the embryo and adult rat. It supports the survival of motor neurons *in vitro* and *in vivo* after sciatic nerve axotomy. It also promotes the survival of ventral midbrain dopaminergic neurons in culture and prevents their degeneration *in vivo* after 6-hydroxydopamine (6-OHDA) treatment. As with GDNF, PSP potentiates kidney morphogenesis, but unlike GDNF and NTN, it does not support any of the peripheral sensory, sympathetic, or enteric neurons.[66] In addition, in contrast to GDNF and NTN, PSP does not protect cultured rat spinal cord motor neurons from toxicity of incubation with threohydroxyaspartate (strong glutamate transport inhibitor producing glutamate-mediated motor neuron degeneration relevant to ALS) and may potentiate the toxicity.[71,72]

ARTN is highly expressed in the pituitary, placenta, trachea, and in human fetal tissues, kidney, and lung.[67] Its expression is generally low in fetal and adult human brain, but present in the basal ganglia and thalamus. Rat embryos show ARTN expression in DRG nerve roots and in Schwann cells in culture. Although not visibly expressed in rat embryonic ventral midbrain, ARTN supports the survival of dopaminergic midbrain neurons in culture. After sciatic nerve transection, ARTN is upregulated in distal nerve segments. As with GDNF and NTN, ARTN is involved in the *in vitro* maintenance of a subset of sensory neurons from the DRG, trigeminal ganglion, and nodose ganglion, in addition to visceral sensory and superior cervical ganglion neurons, and acts as a survival factor for sensory and sympathetic peripheral neurons in culture. ARTN has also been shown to induce a strong differentiation response in a human neuroblastoma cell line. Thus, all of its tested biologic responses suggest that ARTN utilizes receptor components in a manner similar to GDNF and NTN.[67]

GDNF Family Receptors

The GDNF family receptors (GFR) comprise a receptor complex of the RET proto-oncogene product and one of four subtypes of GFRα (α1–α4) [table I]. The GDNF family ligands (GFL) first form a high-affinity complex with one of the four GFR proteins. The complex, containing GFL and GFR homodimers, then brings two molecules of RET together, triggering transphosphorylation of specific tyrosine residues in their tyrosine kinase domains and intracellular signaling.[73] Although GFL act through the same receptor complex (RET/GFR), GDNF has a marked preference for GFRα1, and NTN, ARTN, and PSP specifically bind to GFRα2, GFRα3, and GFRα4, respectively.[74-76] Recent data indicate that GDNF signaling is more complicated than originally thought. It was found that GDNF-GFRα signaling makes use of other receptor systems. In RET-deficient cell lines and primary neurons, GDNF triggers Src-family kinase activation and phosphorylation of extracellular signal-regulated kinase (ERK)/mitogen-activated protein (MAP) kinase, phospholipase-C (PLC)-γ and the transcription factor cAMP response-element binding protein (CREB), and induction of the transcription factor FOS.[77,78] GDNF partially restores ureteric branching morphogenesis in RET-deficient mice that exhibit severe renal hypodysplasia.[79] Additionally, in many areas of the nervous system, and especially in the forebrain, cortex, and inner ear, GFRα receptors are much more widely expressed than RET.[80-82] This suggested that GFLs signal in neuronal and glial cells independently of RET in collaboration with other transmembrane proteins. Ibañéz and coworkers[83] noticed that the signaling pathways triggered by GDNF in an RN33B cell line expressing GFRα1, but not RET, significantly overlap with those triggered by neural cell adhesion molecule (NCAM). They have now demonstrated that NCAM functions as an alternative signaling receptor for GFLs.

Furthermore, new molecular mechanisms of action of GDNF have been described recently.[84] They found that GDNF-induced increases in dopamine function are associated with a sustained increase in tyrosine hydroxylase phosphorylation at Ser31 by a long-term increase in ERK 1/2 signaling after GDNF. In GDNF-treated rats, tyrosine hydroxylase phosphorylation at Ser31 increased 40% in striatum and 250% in the SNpc, one month following a single striatal administration of GDNF. ERK1 significantly increased in phosphorylation in the SNpc. In the striatum, there was a significant increase in ERK2 phosphorylation. Based on descriptive studies outlining the sequence of molecular events associated with GDNF, binding to the GFRα1 receptor activates the RET protein kinase. This activation triggers a cascade involving Ras, Raf, MEK-1 (increase in phosphorylation), and ERK (increase in phosphorylation), resulting in an increase in phosphorylation of tyrosine hydroxylase at Ser31 by ERK1 or ERK2. Therefore, these mechanisms may underlie, in part, the findings that GDNF increases dopamine function in association with motor function recovery in animal models of aging and PD, and more recently, in patients with advanced PD.[85]

CNS Distribution

Immunostaining studies of the human nervous system report that GDNF and its receptor component RET are found in the human peripheral nerve axons and the DRG neurons, and that GDNF is found in Schwann cells.[86] In addition, recently it was

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demonstrated that GFRα1 is expressed in the rat striatum during postnatal development.[87] Experimental studies carried out with RT-PCR techniques indicate that GDNF and its receptor components are broadly expressed in the rat and human hippocampus from early embryonic ages to adult life.[88,89] Available quantitative data show that, in the rat hippocampus, levels of GDNF mRNA[90] and protein[91] are steadily increased from the early embryonic stages to birth, reaching a maximum during the first week after birth and gradually decreasing to prenatal levels in the adult. The occurrence and pattern of overall distribution of GDNF-positive neurons in human hippocampus appeared consistently similar throughout life stages from 29 weeks of gestation to adulthood. However, the relative numerical density of labeled neurons changed with age.[92] GDNF immunostaining was also found in melanin-containing neuronal perikarya of the SNpc in normal human brain.[55,56]

1.3 Neurokine Superfamily

The neurokine family, also termed neuroregulatory cytokines or neuropoietins, includes ciliary neurotrophic factor (CNTF),[93] leukemia inhibitory factor (LIF),[4] interleukin (IL)-6,[94] cardiotrophin-1 (CTF1), and oncostatin-M[10,95] (figure 1). All family members are related to the cytokines and are characterized by similar α-helical tertiary structures and similar signaling pathways via cell surface receptors, namely, leukemia-inhibitory factor receptor (LIFR)[95] and gp130[96] (table I). Neurokines regulate several cell properties in the developing and mature nervous system *in vitro*,[47] including neurotransmitter phenotype, neuronal and glial differentiation and development, and rescue of neurons from axotomy-induced cell death.[95,97]

1.3.1 Identification and Role of Ciliary Neurotrophic Factor (CNTF)

CNTF is the main factor from the neurokine family that acts in the CNS. CNTF is synthesized by astrocytes and is generally considered to be an autocrine and paracrine signal of astrocytic activation and hypertrophy in response to injury in the intact CNS.[98] In the periphery, CNTF is synthesized in muscle and then released for uptake by motor neuron terminals and transported in a retrograde manner to the cell body. Its levels were found to be decreased in the human sciatic nerve during motor neuron disease.[99]

1.3.2 CNS distribution

CNTF was originally identified as a survival-promoting activity for ciliary ganglion neurons in chick eye tissues.[100,101] Subsequently, it was cloned from mammalian nervous tissue.[102-104] The protein is mainly expressed in glial cells of the peripheral nerve system and CNS[105] and has been implicated in many developmental processes in the nervous system, including cell fate determination of neural stem cells, the proliferation and transmitter choice of neuronal precursor cells, and the survival and differentiation of developing neurons as well as glial cells.[106] In addition, it acts on a variety of mature peripheral and central neurons preventing injury-induced cell death and degeneration.[106] Demonstrations of retrograde axonal transport of radiolabeled CNTF in the sensory neurons of the sciatic nerve[107] suggest that CNTF may function by target synthesis and retrograde transport in central neurons. There are no published data on the distribution of CNTF in human brain. However, in rat brain, immunoreactive gp130 has been localized in glial cells and neurons, distributed in the neuropil of telencephalic structures and neuronal somata of the brainstem and spinal cord, and in oligodendrocytes and the subependymal zone.[108] The expression of CNTF mRNA in primates is widely distributed in CNS motor, sensory, and autonomic neurons.[109]

1.4 Non-Neuronal Growth Factors

Non-neuronal growth factors are present in large concentrations in the nervous system and include mainly acidic fibroblast growth factor (aFGF), also called FGF-1, basic fibroblast growth factor (bFGF), also called FGF-2, epidermal growth factor (EGF), and insulin-like growth factors (IGFs) [figure 1]. The concentrations of aFGF are 500-fold greater than NGF, and 50-fold greater than bFGF.[58] FGF binds with low affinity to a heparin sulfate proteoglycan FGF receptor, which then facilitates binding of FGF monomers to the high-affinity FGF receptor. Walker et al.[110] have compared the distribution of FGF-1 receptor (FGFR-1) in post-mortem brain from non-demented young, non-demented old, and PD subjects. A decrease in the intensity of FGFR-1 immunoreactivity in the SNpc neurons between young and old subjects was observed. At the cellular level, most of the immunoreactivity was noted in the perikarya and weak staining seen in neuronal fibers. This topic will not be discussed in the present review (except for IGFs) because of the relatively sparse data in literature.

1.4.1 Insulin-Like Growth Factors (IGF) Family

IGF-1 has a well recognized role as a trophic and survival factor for nervous system cells in tissue culture. However, its specific functions in the developing and mature nervous system have been difficult to define, largely because their trophic factor activities overlap extensively with those of the other growth factors. The IGF system involves complex regulatory networks that operate throughout the organism at cellular and subcellular levels. Key

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molecules involved are the ligands IGF-1 and IGF-2, the type 1 and type 2 IGF receptors (IGF-1R and IGF-2R, respectively), the IGF-binding proteins (IGFBPs), and the proteins involved in intracellular signaling distal to IGF-1R, which include members of the insulin-receptor substrate (IRS) family, AKT (also known as protein kinase B), target of rapamycin (TOR) and S6 kinase.[111-113]

Identification of IGF-1

IGF-1 has characteristics of both a circulating hormone and a tissue growth factor. Most IGF-1 found in the circulation is produced by the liver. The regulation of hepatic IGF-1 production is complex. The growth hormone has a dominant role in upregulating IGF-1 gene expression, but its stimulatory influence is markedly reduced by malnutrition.[114] IGF-2 is also expressed both in the liver and in extrahepatic sites, but is not tightly regulated by growth hormone.

CNS Distribution of IGF-1

IGF-1 is expressed in nervous tissue late in development and is present in its highest concentrations in neurons of the olfactory bulb, thalamus, cerebellum, and retina.[115] Moreover, IGF-1 is a potent neurotrophic as well as neuroprotective factor found in all brain regions in the adult rat including cerebral cortex, hippocampus, and nerve fiber paths. Virtually all brain regions possess IGF binding sites, IGF-2R being ubiquitously expressed and IGF-R localized to neurons of the forebrain, hippocampus, choroid plexus, and meninges.[116-121] In addition, the SNpc is one of the regions in the human brain where a considerable density of IGF-1R is evident[122] and IGF-1 increases the survival of neurons in the brain stem including the SNpc.[119] Transport of peripheral IGF across the blood-brain barrier is accomplished by receptor-mediated transport across endothelial cells.[123,124]

2. Crosstalk Between NTFs

2.1 Neurokine and NGF Families

Several studies have shown that multiple populations of neurons, including the trigeminal, dorsal root, vestibular, cochlear, and superior cervical ganglion, require more than one trophic factor over the course of their development for survival *in vivo*. Hashimoto et al.[125] reported that CNTF and BDNF have synergistic effects on the survival of basal forebrain cholinergic neurons cultured from 2-week-old rats; CNTF enhanced BDNF-mediated promotion of cell survival of cultured cholinergic neurons, (from basal forebrain cholinergic neurons of 2-week-old rats), when added concomitantly. BDNF alone induced only a 3-fold increase in ChAT activity in control cultures, but the concomitant addition of CNTF resulted in an 8-fold increase, although their common signaling mechanism was unclear. However, as CNTF also enhances NTF5 activity, thereby suggesting a role for TrkB, LIF enhances BDNF-mediated protection. Thus, it was proposed that both TrkB and LIFR receptors are involved in this neurotrophic synergism. Other interactions between NTFs, e.g. LIF and the CNTF, might also play a role in preventing the loss of p75 immunostaining in medial septal neurons after fimbria-fornix axotomy.[126]

Chronic CNS injury is associated with tissue scarring and the formation of cavities or cysts that must be bridged with a growth permissive matrix if circuit reconstruction and, ultimately, functional recovery is to occur. Loh et al.[127] described the combined use of BDNF and CNTF neurotrophic therapy in an attempt to promote axon regeneration across a CNS tissue defect. The large, statistically significant increase in the density of axons in implants that contained both BDNF- and CNTF-expressing fibroblasts, many of these axons most likely originating from neurons in the adjacent diencephalic or midbrain regions, suggests that diverse types of CNS neurons may be responsive to the combined application of these factors. It is most likely that synergism occurs because BDNF signals through TrkB and p75 receptors, whereas CNTF binds to a heterotrimer receptor (comprising CNTFRα, LIFRβ subunit, and gp130) and activates different cell signaling pathways that modulate different subsets of genes.

Analysis of the cerebrospinal fluid (CSF) of patients after severe brain trauma revealed high levels of IL-6; NGF appeared in the CSF only when IL-6 levels were elevated. Accordingly, NGF release was induced by IL-6, in astrocytes, and this induction was blocked by adding anti-IL-6 antibodies.[128]

2.2 GDNF and NGF Families

Giehl et al.[129] reported that the survival-promoting effect of GDNF on axotomized corticospinal neurons *in vivo* is mediated by a BDNF mechanism. On *in situ* hybridization, almost all the corticospinal neurons expressed mRNA for TrkB, but only about half expressed mRNA for BDNF. Furthermore, GDNF rescued corticospinal neurons after axotomy only when endogenous BDNF was not neutralized by antibodies. In contrast, NTF3 promoted the survival of axotomized corticospinal neurons even when BDNF was neutralized. These findings indicate a cross-talk between BDNF and GDNF, in addition to the potential for compensatory mechanisms when one factor is blocked or deficient.[129]

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One of the most severely affected neuronal populations in GDNF knock-out mice is the nodose-petrosal ganglion complex (NPG) of primary cranial sensory neurons, in which 40% of cells die by birth.[130] Interestingly, targeted disruption of the genes encoding BDNF, NTF3 or 4 also leads to loss of 30–50% of NPG neurons, suggesting that some neurons require both GDNF and a neurotrophin for survival *in vivo*. However, it was unknown whether GDNF and the neurotrophins act simultaneously or sequentially.

To consider these issues Erickson et al.[131] defined the trophic requirements of NPG neurons for BDNF and GDNF by comparing ganglion cell numbers in wild-type mice and in mice, lacking genes for either BDNF or GDNF, or both BDNF and GDNF. In addition, to examine the relationship between GDNF dependence and target innervation, they analyzed survival requirements of different ganglion cell subpopulations, including dopaminergic neurons in the petrosal ganglion (PG) that selectively innervate the carotid body, as well as the distribution of GDNF protein in target tissues. It was found that neuron losses in BDNF/GDNF double mutants are not additive to the losses in single BDNF or GDNF null mutants, indicating that many cells, including dopaminergic neurons, require both GDNF and BDNF for survival *in vivo*. Moreover, both factors are required during the same period of development, between embryonic day (E) 15.5 and E17.5. Furthermore, because some further cell loss was observed in BDNF-null mice before E14.5 and after E17.5, the possibility cannot be ruled out that some cells, initially dependent on BDNF, switch their dependence to GDNF and vice versa. In addition, GDNF, like BDNF, is expressed in target tissues at the time of initial target innervation and coincident with GDNF dependence on the innervating neurons. Together, these findings demonstrate that both GDNF and BDNF can act as target-derived trophic factors and are required simultaneously for the survival of some primary sensory neurons. There are several potential mechanisms that could explain the simultaneous dependence of some PG neurons on BDNF and GDNF. One possibility is that both factors are able to independently support survival, but are present *in vivo* at concentrations below the threshold for either factor alone to be effective. This hypothesis was supported by the finding that BDNF or GDNF can each support survival of PG neurons in culture and that at subsaturating concentrations,[131] the combination of the two factors results in an increase in survival. Another option is that, *in vivo*, only one of the two factors acts directly to support survival, and the other regulates access or responsiveness to the other.

A different study investigated whether local expression of BDNF or GDNF near neuronal cell bodies of the intact corticospinal tract enhanced axonal sprouting induced by concomitant overexpression of NTF3 in the lumbar spinal cord.[132] The results clearly indicated that NTFs expressed at different sites increase axonal plasticity after spinal cord injury. The simultaneous delivery of NTF3, BDNF, and GDNF might improve the outcome achieved with double neurotrophic factor therapy, if the latter two factors act through independent pathways.

In an *in vitro* study, cultured ventral mesencephalic neurons, many of which co-express RET and TrkB, both BDNF and GDNF induced rapid phosphorylation of mitogen-activated protein kinase (MAPK) and of the cAMP-response element-binding protein (CREBP). However, their kinetic and pharmacologic effects were different. GDNF-induced phosphorylation of MAPK was decreased and was completely blocked by a specific inhibitor of the MAPK kinase (MAPKK) that did not block BDNF-induced phosphorylation of MAPK. While both GDNF and BDNF induced phosphorylation of CREBP, GDNF, but not BDNF, phosphorylation was blocked by the same MAPKK inhibitor. Thus it appears that these two trophic factors may recruit the same secondary messengers, but they do so through different pathways which may mediate distinct biologic functions.[133] Certain differences in kinetics and pharmacologic interventions on protein phosphorylation levels may also arise from differential activation or inhibition of phosphatases.

3. NTFs in Neurodegenerative Diseases

3.1 Parkinson Disease

PD is a neurodegenerative disorder of an unknown cause that affects >1 million people in North America.[134] Age is the single most consistent risk factor, and with the rising age of the general population, the prevalence of PD will escalate in the future. The impact of the disease is indicated by the fact that mortality is 2- to 5-fold higher among affected patients than age-matched controls,[135,136] resulting in a marked reduction of life expectancy.[136] Thus, PD greatly shortens duration as well as quality of life.

The classic triad of major signs of PD is made up of tremor, rigidity, and akinesia.[137] The diagnosis of PD is made on the basis of clinical criteria. The hallmark for the diagnosis of PD remains the neuropathologic examination. There is still no biologic marker that unequivocally confirms the diagnosis. PD is characterized by the progressive death of selected but heterogeneous populations of neurons, including the neuromelanin-laden dopaminergic neurons of the SNpc, selected aminergic brain-stem nuclei (both catecholaminergic and serotoninergic), the cholinergic nucleus basalis of

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Meynert, hypothalamic neurons, and small cortical neurons (particularly in the cingulate gyrus and entorhinal cortex), as well as the olfactory bulb, sympathetic ganglia, and parasympathetic neurons in the gut.[134] Not all dopaminergic projection areas are equally susceptible. Within the SNpc, neuronal loss tends to be greatest in the ventrolateral tier (loss is estimated to be 60–70% at the onset of symptoms), followed by the medial ventral tier and dorsal tier.[138] This pattern of cell loss is relatively specific to PD; it is the opposite of that seen in normal aging and differs from patterns found in striatonigral degeneration and progressive supranuclear palsy. It results in a regional loss of striatal dopamine, most prominently in the dorsal and intermediate subdivisions of the putamen,[139] a process that is believed to account for akinesia and rigidity. Another important pathologic feature is the presence of degenerating ubiquitin-positive neuronal processes or neurites (Lewy neurites), which are found in all affected brain-stem regions, especially the dorsal motor nucleus of the vagus.[140] Other possible clinical-pathologic correlations include neurodegenerative changes in the olfactory bulb causing anosmia; degeneration in the intermediolateral columns of the spinal cord, sympathetic, and parasympathetic ganglia, and possibly the central amygdaloid nucleus[141] causing autonomic dysfunction; and degeneration in the brain-stem serotoninergic and noradrenergic nuclei and possibly the amygdaloid nucleus causing behavioral dysfunction, including depression, which occurs in approximately one quarter of patients.[142]

The mechanisms responsible for cell death in PD are largely unknown. Increasing evidence suggests that neuronal death in the SNpc may be apoptotic,[143] but this notion is not universally accepted.[144] Among the factors that have been implicated in neuronal degeneration in PD are mitochondrial dysfunction, oxidative stress, the activity of excitotoxins, deficient neurotrophic support, and immune mechanisms.[134] A critical question is why specific neurons are selectively vulnerable in PD. One possible answer may lie in their ability to take up both endogenous and extrinsic toxic compounds through selective carrier mechanisms, such as the dopamine transporter.[134] Other possible explanations include increased metabolic stress, high physiologic rates of protein oxidation, selective generation of potential toxins or failure to detoxify or dispose of them (possibly because of the presence of neuromelanin), and specific requirements for neurotrophic support.[134]

3.1.1 NGF Family

As mentioned before, the members of the NGF family and their receptors are localized in melanin-positive neurons in the SNpc. In a quantitative assessment of NGF family content within individual neurons in the SNpc and striatum of patients with PD, researchers observed a small decrease in staining density for BDNF and NGF,[145-147] but little or no significant changes in staining for NTF3 or NTF5.[55,56]

With regard to NGF family receptors, an *in situ* hybridization study of TrkB mRNA failed to show any change in the mRNA content per surviving neurons in the SNpc of PD patients despite the extensive reduction in the total number of neurons.[148] BDNF levels were more reduced in non-BDNF-immunoreactive pigmented neurons, suggesting that the pigmented neurons in the SNpc that do not express BDNF have a greater probability of surviving than BDNF-positive pigmented neurons.[149]

3.1.2 GDNF Family

Many studies have demonstrated that GDNF control the survival and physiologic properties of mesencephalic dopaminergic neurons both *in vitro* and *in vivo*.[150-154] Therefore, reduction of GDNF expression in the SNpc, leads to neuronal loss in PD.[151-154]

Although one early *in situ* hybridization study of GDNF mRNA expression failed to show any labeling in the mesencephalon and striatum of other control subjects or patients with PD,[155] Schaar et al.,[156] using PCR, reported the highest expression of GDNF mRNA in the human caudate, low levels in the putamen, and no detectable mRNA in the substantia nigra. Springer et al.[89] observed GDNF transcripts in human striatum, hippocampus, cortex, and spinal cord, but not in cerebellum. GDNF transcripts were found in all rat regions of the rat CNS and in a Schwann cell line that secretes a dopaminergic NTF.[110] Apparently, *in situ* hybridization, in contrast to PCR, does not produce labeling in human brain[155] or rodent brain, except in DRG of 1-day-old rats.[89] Hence, PCR amplification should be used for brain analyses of GDNR-mRNA expression in PD. Receptor studies found that tissue sections from the SNpc of control subjects and PD patients show localized RET immunoreactivity in the dopaminergic neurons, appearing as punctuate deposits within cells.[110] RET mRNA expression was also observed in many surviving neurons in all PD patients. In addition, GDNF is present in the adult striatum and the dopaminergic neurons of the SNpc express mRNAs encoding the GDNF receptors, RET and GFRα1.[157] Chauhan et al.[55,56] found that GDNF levels were significantly reduced (about 20%) in all sampled surviving melanin-containing neurons in the SNpc from PD patients compared with age-matched controls.

Indeed, Bozzi et al.[158] found a marked reduction of GDNF mRNA in the dopaminergic system of D2 dopamine receptor (DRD2) knockout adult mice compared with wild-type litter

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mates. These results implicate dopamine, acting through D2 receptors, in the local control of GDNF expression. The downregulation of GDNF expression might also contribute to the locomotor phenotype of DRD2 −/− mice. Reduced levels of GDNF might be detrimental for the physiologic properties of striatal and nigral neurons.

3.1.3 Neurokine Family

The distribution of CNTF in SNpc of PD brains has been investigated using immunofluorescence in a case study that included four 69- to 77-year-old neurologically normal male controls and four 72- to 79-year-old male PD patients. The results showed 56% loss of SNpc neurons in PD brains compared with age-matched controls. Despite considerable neuronal dropout, immunofluorescent NTFs in the PD brains showed differential reductions that were consistent within the group as compared with age-matched controls: reductions were GDNF, 19%/neuron, 20%/neuropil; CNTF, 11.1%/neuron, 9%/neuropil.[54] These single observations suggest a role for decreased availability of CNTF in the process of SNpc neurodegeneration in PD.

Several neurokines, including IL-6, were found to be increased in the striatum, but not the cerebral cortex of brains from PD patients.[146] The concentration of proinflammatory agent, IL-6, increases in the striatum during the early stages of PD, which correlate with the severity of this disease.[159] In patients with PD, plasma concentrations of IL-6 increase to a level observed in people 10 years older.[160] Significantly elevated levels of IL-6 were found in the CSF of PD patients. Moreover, a significant inverse correlation between severity of PD and IL-6 CSF levels appeared.[161]

Nagatsu et al.[162,163] found markedly increased levels of cytokines, such as tumor necrosis factor (TNF)-α, and IL-6 in the nigrostriatal DA regions and ventricular and lumbar CSF of PD patients. Furthermore, the levels of TNFα receptor R1 were also elevated in the nigrostriatal DA regions in PD. In experimental animal models of PD, TNFα level was increased in the SNpc and striatum of the 6-OHDA-injected side of hemiparkinsonian rats. Increased levels of proinflammatory cytokines, cytokine receptors, and caspase activities, and reduced levels of neurotrophins in the nigrostriatal region in PD patients, and in parkinsonian animals, suggest increased immune reactivity and programmed cell death (apoptosis) of neuronal and/or glial cells.[162,163] In addition, reactive gliosis, the cellular manifestation of neuroinflammation, is a pathologic hallmark of neurodegenerative diseases, including PD. It was found that IL-6 regulation of microglial activation and the persistent gliosis observed in the PD SNpc in humans and in PD animal models may represent a chronic inflammatory response that contributes to pathology.[164] Changes in lymphocyte populations in CSF and blood, immunoglobulin synthesis, and cytokine and acute phase protein production have been observed in PD patients.[165] The balance between the increased levels of pro-inflammatory cytokines and the decreased levels of neurotrophins requires further investigation with respect to the molecular mechanisms of PD.

3.2 Aging and Mild Cognitive Impairment

Aging appears to be the result of normal developmental and metabolic processes that are responsible for many of the pathophysiologies that increase the chance of disease, especially neurodegenerative diseases, and death.

There are only minimal data available on changes in the expression of any of the NTFs or their receptors during aging. In the human hippocampus, the area of BDNF immunostaining increased with age, whereas staining for NGF, NTF3, and NTF5 was generally higher at earlier ages.[61]

Aging in the adult brain is accompanied by significant and quantitatively extensive neuronal atrophy in subcortical cholinergic neuronal populations. Further, these spontaneous age-related changes in subcortical cholinergic neurons represent atrophy rather than death, because they are reversible by the delivery of the NGF gene at a moderate stage of aging.[166] An alternative mechanism of NGF-induced increases in chergic cell numbers in the aged primate brain may be postulated: neurotrophin delivery induces neurogenesis in the cholinergic basal forebrain. In addition, it is now well known that neurogenesis takes place in the adult vertebrate brain, residing primarily in the ventricular/subventricular zone (SZ), and that it is dependent, at least in part, on specific NTF levels.[166-168] However, this possibility is unlikely, because previous studies of neurogenesis in the mammalian brain have not demonstrated neuronal precursor cells in the cholinergic basal forebrain. This finding was further supported by *in vitro* studies showing that the serial application of BDNF is associated with the generation and maintenance of neuron precursor cells arising from the adult human SZ[168] and not from cholinergic basal forebrain. These data suggest the induction of neurogenesis in the adult human ventricular/SZ with appropriately selected and/or regulated NTFs is feasible *in situ* or with implanted progenitor cells.[168]

An increasing number of studies clearly indicate that the onset of AD is typically preceded by a prodromal phase of mild cognitive impairment (MCI). While MCI can affect many areas of cognition, such as language, attention, critical thinking, reading,

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and writing, most research has focused on its effects on memory.[169] The disorder can be divided into two broad subtypes: amnestic MCI, significantly affecting memory; and nonamnestic MCI, which does not. Other functions, such as language, attention, and visuospatial skills, may be impaired in either type. Most importantly, the diagnosis of MCI relies on the fact that the individual is able to perform all their usual activities successfully, without more assistance from others than they previously needed.[169]

How do the memory difficulties in MCI differ from those of normal aging? This is a very difficult question to which there is, as yet, no definitive answer. Several studies have examined the cognitive performance of patients with MCI. These have demonstrated that, in general, these patients perform relatively poorly on formal tests of memory, even when compared with other individuals in their age group. They also show mild difficulties in other areas of thinking, such as naming objects or people (coming up with the names of things) and complex planning tasks. These problems are similar, but less severe, than the neuropsychologic findings associated with AD.[169]

MCI is a transitional stage between normal aging and dementia, and is characterized primarily by memory deficit without clinically meaningful functional impairment. Recent data suggest that patients with MCI have up to a 50% probability of progressing to symptomatic AD within 4 years, for a rate of progression of approximately 12% per year.[170] One study of individuals with MCI and early AD reported a loss of nucleus basalis neurons containing TrkA immunoreactivity.[171] However, a later study performed by the same group showed no change in cortical or hippocampal levels of NGF in patients with MCI.[172]

3.3 Alzheimer Disease

AD is a progressive neuropsychiatric disorder of unknown etiology. It is characterized by neuronal degeneration and cognitive deterioration, especially in the elderly. The deterioration in cognition correlates with a number of pathologic changes in several different brain regions, particularly the basal forebrain cholinergic neurons.[173] In addition, AD is the most common form of dementia in the elderly. Dementia is commonly recognized as criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV).[174,175] The classic clinical features of AD are an amnesic type of memory impairment,[176,177] deterioration of language,[178] and visuospatial deficits.[179,180] Motor and sensory abnormalities, gait disturbances, and seizures are uncommon until the late phases of the disease.[181] Behavioral disturbances also progress over the course of the illness.[175] Mood change and apathy commonly develop early and continue for the duration of the disease. Psychosis and agitation are characteristic of the middle and later phases of the disease.[182]

There is increasing consensus that the production and accumulation of β-amyloid (Aβ) peptide is central to the pathogenesis of AD.[183] Evidence supporting a pivotal role for Aβ includes the following: mutations in the amyloid precursor protein lead to early-onset AD; all currently known mutations associated with AD increase the production of Aβ. Formation of neurofibrillary tangles, oxidation and lipid peroxidation, glutamatergic excitotoxicity, inflammation, and activation of the cascade of apoptotic cell death are considered secondary consequences of the generation and deposition of Aβ.[183]

3.3.1 NGF

Both gene expression and protein levels of NGF have been investigated in AD. Support for the role of NGF in AD was provided by several experimental studies in rats wherein severing the fimbria fornix to disrupt the connection between the septum and the hippocampus, prevented the retrograde transport of NGF via cholinergic fibers to the cell bodies and resulted in cholinergic cell atrophy.[184-188] In addition, knockout mice lacking both NGF and TrkA showed large reductions in ChAT immunoreactivity in the basal forebrain and loss of cholinesterase activity in both the hippocampus and cortex.[59]

Several studies reported a widespread increase in NGF in cortical and subcortical brain regions in AD.[189-192] The precursor of NGF (proNGF) is the predominant form of NGF in the brain. Recently, Peng et al.[193] measured the relative amounts of proNGF protein in the parietal cortex from subjects clinically classified with no cognitive impairment, MCI, or mild to moderate AD. It was found that proNGF increased during the prodromal stage of AD. The amount of proNGF protein was 1.4-fold greater in the MCI group than in the no cognitive impairment group, and was 1.6-fold greater in mild-moderate AD than in the no cognitive impairment group. There was a negative correlation between proNGF levels and Mini-Mental Status Examination (MMSE) score, demonstrating that the accumulation of proNGF is correlated with loss of cognitive function. However, others found that the levels of NGF were unchanged[194,195] or decreased.[196] Accordingly, studies of NGF gene expression provided no evidence for upregulation of this gene in AD.[197-199] A similar discrepancy was noted for TrkA protein or TrkA mRNA levels in regions of the cortex and within surviving cholinergic basal forebrain neurons (reduced or no change)[14,200-203] and for p75 expression.[201,204]

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Biochemical measurements showed that TrkA protein level was reduced in the nucleus basalis of brains from AD patients, but unaffected in the caudate nucleus. Nuclear translocation of NF-γ B, which could be a response to p75 in cholinergic neurons, was increased in cholinergic nuclear basalis of Meynert neurons in AD.[205] However, in immunocytochemical studies, the proportions of neurons expressing TrkA, TrkB, and TrkC in the nucleus basalis of Meynert in AD patients was lower than in controls.[206]

3.3.2 Brain-Derived Neurotrophic Factors

Several studies found a decreased transcript abundance of BDNF mRNA in the hippocampus, especially the pyramidal layer of CA1-CA4 subfields, and entorhinal cortex of patients with AD.[6,193,207-209] Murer et al.[51] observed deposits of BDNF-immunoreactive material resembling senile amyloid plaques in the subiculum, entorhinal cortex, and other cortical areas of the brain. Cortical neurons with neurofibrillary tangles were usually devoid of BDNF-immunoreactive material, whereas most of the neurons exhibiting intense BDNF immunostaining did not have neurofibrillary tangles. However, a few immunoreactive pyramidal cell bodies with discrete deposits of fibrillary fluorescent material were found in the apical cytoplasm and proximal apical dendrites. In these cells, BDNF immunoreactivity was present in the rest of the cell body cytoplasm, while the cellular region affected by the tangles showed reduced immunoreactivity.

Taken together, these results show that a role of BDNF dysfunction in the process of neuronal degeneration in AD cannot be ruled out. In particular, the presence of tangles in BDNF-immunoreactive neurons powerfully suggests that these cells degenerate in AD, leading to a reduction of paracrine trophic support of close-by neurons and target-derived support of far-off afferent neurons. It was shown in rats that BDNF could be transported anterogradely by axons.[210] It has also been publicized that depolarization induces the discharge of BDNF from hippocampal neurons in culture.[211] The results of these studies propose that BDNF can be released in a synaptic-like mode from nerve terminals and provide afferent-derived trophic maintainance to target neurons. Therefore, degeneration of BDNF-immunoreactive neurons in the entorhinal cortex or the hippocampus could lead to reduced trophic support of targeted hippocampal cells. Even though the hypothesis that a reduction in BDNF support can contribute to neuronal degeneration in AD seems reasonable, it should be noted that a clear demonstration of a trophic action of endogenous BDNF on adult CNS neurons is lacking.

There is also some evidence to suggest a change in the TrkB receptor in AD. Immunopositive, truncated (95 kDa) TrkB receptors have been identified in amyloid plaques of the hippocampus.[118] In several brain regions, such as the cortex and the basal forebrain, Western blot or immunostaining showed a large reduction in TrkA, TrkB,[209,212] and TrkC expression,[206] although *in situ* hybridization showed no change in TrkB.[205] In the parietal cortex, TrkB mRNA was reportedly unchanged but TrkA was reduced to <50% of normal levels.[193]

Hence, the available data on TrkB receptor expression in glia are conflicting. One group suggested that glial expression of BDNF and TrkB receptors may be characteristic of the particular disease process and not a stereotyped response to any injury.[52]

In summary, the various reports support increases in NGF and decreases in BDNF in the hippocampal and neocortical regions, and decreases in TrkA in the cortex and nucleus basalis in advanced AD.

3.3.3 Neurotrophin-3

Immunoreactive levels of NTF3 were found to be decreased in the cortex of AD patients relative to the corresponding brain regions in non-demented controls.[189,195,196]

As a final point, it should be noted that deposits of other molecules possessing neurotrophic activity (FGF, EGF) or that are preferentially expressed during axonal sprouting (integrins, laminin, growth-associated protein-43) have been found in senile plaques in AD.[213-215] Based on these findings, it has been suggested that abnormal regenerative processes occur in senile plaques, in concert with the primary degenerative mechanism.[216]

3.4 Huntington Disease

HD is an autosomal dominant neurodegenerative disease characterized by a progressive choreic movement disorder. The pathology is restricted to the brain, with atrophy and neuronal loss occurring foremost in the striatum and to a lesser extent in the cerebral cortex. Onset usually occurs during the fourth or fifth decade of life, with a mean survival after onset of 15–20 years.[217] HD is universally fatal, and there is no effective treatment. Symptoms include a characteristic movement disorder (Huntington chorea), uncontrolled movements, loss of intellectual faculties, cognitive abnormalities, emotional disturbances, and psychiatric symptoms.[217]

Genetic studies have shown that HD is caused by expansion of the unstable polyglutamine trinucleotide repeats (CAG repeats) in the coding region (open reading frame of its first exon) of the HD gene, which is located on chromosome 4p16.3.[217,218] Normal subjects have a median of 19 CAG repeats (range, 11–34), whereas nearly all patients with HD have >40.[219,220] Unstable or dynam-

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ic mutations have been identified in a few families, in which one parent has 34–38 CAG repeats and the progeny have >40.[221-223] The HD gene encodes a protein named huntingtin. The increased number of CAG repeats in the HD gene are expressed as an elongated huntingtin protein with 40–150 glutamine residues.[224] The protein, whose function is unknown, is found in many cells in both neural and non-neural tissues.[225] No direct relation between the amount of elongated huntingtin and the extent of neuronal injury has been found. However, the brains of both humans with HD and transgenic mice with increased numbers of CAG repeats have intranuclear inclusions of huntingtin and ubiquitin in neurons of the striatum and cerebral cortex but not in the brain stem, thalamus, or spinal cord, matching closely the sites of neuronal cell loss in the disease.[224]

Overexpression of wild-type huntingtin protein in cell lines and ‘knock-in’ transgenic mice led to increased levels of BDNF mRNA and protein.[226] In contrast, mutant huntingtin downregulated BDNF production.[226] In addition, the level of BDNF protein was reduced in the striatum and cortex of transgenic mice overexpressing mutant huntingtin. Most importantly, in patients with HD, BDNF levels were reduced by 45% in cortical brain tissue.[226]

3.5 Amyotrophic Lateral Sclerosis

The most common motor-neuron disorder is ALS,[227] which usually begins in the fifth and sixth decades of life. In a typical patient, it is characterized by a selective and progressive degeneration of the lower motor neurons in the brain stem and spinal cord that innervated the muscles, and the upper motor neurons in the cerebral cortex. The only recognized treatment for ALS is riluzole, which extends survival by only a few months. The illness is usually sporadic, but in 1–10% of patients it is familial, being inherited as an autosomal dominant trait.[228] The cause of motor-neuron loss in ALS remains unknown, but a subgroup of patients with the familial cases (<20%) have dominant mutations in the Cu/Zn superoxide dismutase type 1 (SOD1) gene on chromosome 21,[229] which encodes a protein involved in the regulation of intracellular free radicals. Speculations regarding the mechanisms of cell death in patients with mutations in SOD1 must account for the finding that many patients have no reductions in the concentration of SOD1.[224] Furthermore, mice with a complete lack of SOD1 survive for at least 18 months without evidence of motor-neuron loss. The consensus is that SOD1 mutations cause a gain of function that is selectively lethal to motor neurons.[224] The clinical history and histologic abnormalities in sporadic and familial forms of ALS are not distinguishable. The prognosis is grave in both forms, with death occurring within 3–5 years in 95% of patients. Neuropathologic studies show loss of motor neurons throughout the neuraxis. The death of neurons is preceded by perikaryal shrinkage, the formation of webs of ubiquitin-positive threads,[227,230] and axonal swellings that stain for ubiquitin and for α-synuclein.[231] Although major advances in recent years have shed light on the etiology of ALS, the key mechanisms in both the familial and sporadic type remain unknown.[232]

3.5.1 NGF Superfamily

One post-mortem study showed that NGF concentrations were 140% higher in muscle biopsy specimens from ALS patients than from controls.[233] Another found increased mRNA and protein levels of NGF, BDNF, NTF3, and NTF5.[234] BDNF was strongly upregulated in the early stage of the disease, whereas levels of NGF, NTF3, and NTF5 gradually increased during the course of the disorder. In spinal cords from ALS patients, TrkB mRNA was upregulated,[235] but the receptor was much less phosphorylated on tyrosine residues, compared with controls.

In the spinal cords of patients who died from ALS, three-quarters of the motor neurons had degenerated, and the remainder demonstrated decreased BDNF and increased NGF and TrkA. These data suggest a switch in regulating in the surviving neurons from mainly BDNF and NTF3 to NGF.[236] This observation has been demonstrated only in a single report and further studies are required to establish switching neurotrophins in ALS.

3.5.2 GDNF

Two studies found increased expression of GDNF in muscle biopsy[237] and CSF specimens[238] from patients with ALS. These findings indicate that ALS is associated with an enhanced capacity to synthesize GDNF. Additionally, semiquantitative RT-PCR analysis revealed that RET mRNA levels in the anterior horn of the spinal cord of ALS patients were reduced to one-fifth of control levels. In proportion to the motor neuron loss; GDNFR-α mRNA levels remained unchanged.[239]

4. Neurotrophic Effects in the Treatment of Neurodegenerative Diseases

This section describes both preclinical and clinical studies involving treatment of neurodegenerative diseases with NTFs. The clinical studies are summarized in table III.

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Table III. Treatment of neurodegenerative diseases by neurotrophic factors: clinical studies

Neurotrophic factor	Human clinical trial	Rescue method	Result	Reference
Parkinson disease				
GDNF	Five PD patients in a phase I safety trial	Chronic infusion of GDNF directly via a pump and catheter into the postero-dorsal putamen	Clinical improvements in off-medication motor symptoms and dyskinesias, increased putamen dopamine storage on PET scans	85
GDNF	Double-blind, placebo-controlled, sequential cohort study in 50 patients with PD	Intracerebroventricular placebo and GDNF for 8 months	No improvement in parkinsonism. Spectrum of adverse effects indicate biologically-active GDNF	240
Alzheimer disease				
NGF	Total of four patients with symptoms of dementia	Intraventricular infusion of NGF over 3 months	Significant increase in nicotine binding in frontal and temporal cortex and a persistent increase in cortical blood flow. Some improvements in a few cognitive tests. Adverse effects included loss of weight and appetite, and significant back pain	241,242
NGF	Eight patients with early-stage Alzheimer disease (phase I open-label study)	Intraparenchymal (nucleus basalis) grafts of autologous fibroblasts genetically modified to secrete human NGF	A year and a half after treatment, participants experienced mental decline at a rate that was slower than before their surgery as well as less than the average expected rate. Imaging with PET showed increased brain activity	243
Amyotrophic lateral sclerosis				
BDNF	6-month phase I/II trial in ALS patients	Subcutaneous BDNF 25 or 100 μg/kg	Increased survival rate and delayed loss of pulmonary function. Adverse effects included injection site reactions, diarrhea (dose-related), bowel urgency	244
BDNF	Multicentre placebo-controlled 9-month trial in 1135 ALS patients	Subcutaneous BDNF 25 or 100 μg/kg	No significant treatment effect, but a trend toward increased survival in high-risk patients	245
BDNF	Double-blind, placebo-controlled, randomized, sequential, dose-escalation study of 25 ALS patients (phase I/II)	Intrathecal infusion BDNF 25–1000 μg/day for 12 weeks	No clinical improvement. Adverse effects included sleep disturbance, dry mouth, agitation (at higher dosages >150 μg/day). However, overall 150 μg/day was well tolerated	246
CNTF	9-month double-blind, placebo-controlled, randomized study in 72 and 730 ALS patients	Subcutaneous CNTF 30 or 15 μg/kg	No clinical improvement. Adverse effects included fatigue, aphthous stomatitis, anorexia, weight loss, asthenia, nausea, injection site reactions, headache, increased salivation, dyspnea, and cough	247-249
CNTF	17 week phase I study in six ALS patients	Intrathecal injection of encapsulated genetically engineered baby hamster kidney cells releasing CNTF	Intrathecal delivery of CNTF was not associated with the limiting adverse effects observed with systemic delivery	250
IGF-1	Double-blind, placebo-controlled, randomized study in 266 and 183 ALS patients	Subcutaneous IGF-1 0.05 or 0.1 mg/kg/day for 9 months	Slowed the progression of functional impairment and the decline in health-related quality of life with no medically important adverse effects	251,252

ALS = amyotrophic lateral sclerosis; **BDNF** = brain-derived neurotrophic factor; **CNTF** = cilliary neurotrophic factor-1; **GDNF** = glial-cell-line-derived neurotrophic factor; **IGF** = insulin-like growth factor; **NGF** = nerve growth factor; **PD** = Parkinson disease; **PET** = positron emission tomography.

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4.1 Parkinson Disease

4.1.1 Experimental Studies with Hydroxydopamine Lesion

NGF family: Two *in vitro* studies showed that BDNF treatment increased the number of dopaminergic neurons and the release of dopamine in human[253] and rat[254] fetal ventral mesencephalon cells. An early *in vivo* study found that low doses of BDNF did not attenuate nigral dopamine loss following medial forebrain bundle transection.[255] However, a later study on BDNF and NTF4/5 in rats with a unilateral 6-OHDA lesion showed evidence of increased dopamine metabolism and turnover, as determined by homovanillic acid and dopamine ratios, and improvement in amphetamine/apomorphine-induced rotation despite the lack of an obvious effect on neuron survival or sprouting.[256] Accordingly, BDNF treatment, combined with a neural graft, greatly enhanced the reinnervation of the host striatum by the engrafted dopamine neurons, as determined by tyrosine hydroxylase immunostaining, and also increased the effect of the graft on locomotor behavior induced by amphetamine administration.[257] Furthermore, transplantation of BDNF-transduced astrocytes into the striatum of 6-OHDA-lesioned animals did not enhance dopamine neuron survival, although it significantly reduced amphetamine-induced rotation.[258] Levivier et al.[259] showed that intrastriatal grafts of fibroblasts, genetically engineered to produce BDNF, partially prevent the loss of nerve terminals and completely prevent the loss of cell bodies in the nigrostriatal dopaminergic pathway induced by 6-OHDA in rats. In a rat model, wherein ibotenic acid was injected into the striatum/ pallidus, supranigral infusions of BDNF (but not NTF3) not only supported the dopaminergic neurons, but also prevented the loss of presumed GABAergic neurons in the SNpc. Since ibotenic acid causes excitotoxic neuronal destruction, these data suggest that BDNF can diminish the excitotoxic effects of glutamate in the SNpc.[260]

Several studies used the adeno-associated virus to deliver BDNF into the brain. For example, Klein et al.[261] showed that long-term, stable expression of BDNF in recombinant adeno-associated virus vector injected into the SNpc can modulate locomotor activity without significantly affecting nigrostriatal dopaminergic survival. Wang et al.[262] reported that implantation of normal neonatal rat astrocytes that were co-transfected with a vector expressing BDNF (AAVBDNF) and a retroviral vector expressing tyrosine hydroxylase into the striatum of 6-OHDA-lesioned rats significantly improved PD-like symptoms.

According to these mixed results, more studies are needed in order to determine unequivocally whether BDNF is in fact effective in diminishing PD-like symptoms

GDNF family: The finding that GDNF increases the survival and differentiation of dopaminergic neurons in cultures of fetal midbrain cells,[150,263,264] prompted researchers to investigate its potential protective effect in 6-OHDA-lesioned rodent and primate models of PD. The influence of many variables on behavioral and biochemical parameters were examined: age of the rats, type of administration (single or continuous), time of administration (before or shortly after insult), place of injection (e.g. striatum, brain parenchyma), and method of delivery into the brain (e.g. microspheres, vectors). Most of the studies showed that GDNF ameliorated the behavioral and pathologic consequences of 6-OHDA lesioning.[265,266] Recombinant GDNF protein rescued injured or axotomized dopamine neurons and preserved injured, atrophic dopamine neurons during chronic neurodegeneration.[151,153,267-270] It also stimulated regenerative growth or axonal sprouting after partial lesions of the dopamine system and stimulated the metabolism and function of lesioned dopamine neurons.[271-273]

Studies using adenoviral, adeno-associated viral or lentiviral vectors expressing GDNF in the 6-OHDA-induced degeneration model reported that viral delivery of a GDNF gene with several cell lines protected or rescued dopamine neurons.[154,274-279]

Injections of NTN in the SNpc are shown to be effective in protecting nigral neurons after intrastriatal 6-OHDA lesions.[70,280,281] In the adult rat striatum subjected to 6-OHDA lesions, GDNF administration either into the striatum or into the cerebral ventricles produces about 92% protection of the lesioned dopaminergic nigral neurons, while NTN administration intrastriatally is 72% effective and totally ineffective after intracerebroventricular (ICV) injection. This suggests that within the striatum, NTN is a less protective agent than GDNF. This difference might be related to solubility.[70] Another member of the family, such as PSP,[282] was also shown to prevent the loss of dopamine neurons and improve the behavioral impairment of 6-OHDA-lesioned rodents.

4.1.2 Experimental Studies with MPTP

NGF family: In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) experimental models of PD, BDNF promoted the survival of mesencephalic dopaminergic neurons in rats[283] and monkeys.[284,285] Intravenous injection of NGF, conjugated to transferring, in MPTP mice prevented the loss of tyrosine hydroxylase-immunoreactive neurons located in the SNpc and elevated cell counts of NGF.

GDNF family: GDNF injected over the SNpc or into the striatum before or after MPTP in mice[152,286] and

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monkeys[153,266,287-289] potently protected the dopamine system, as indicated by the number of mesencephalic dopamine nerve cell bodies, density of dopamine nerve terminals, levels of dopamine, fiber densities, and motor behavior. Several studies that used adenoviral, adeno-associated viral, or lentiviral vectors expressing GDNF reported dopamine-cell protection against MPTP in rodents[290,291] and monkeys.[292] A combined BDNF and GDNF treatment was also found to have a beneficial effect on fetal nigral tissue.[264,293]

4.1.3 Clinical Studies with GDNF

The potential of GDNF as a therapeutic agent in PD stems from its ability not only to provide symptomatic relief but also to modify the disease state. In 1999, Kordower et al.[294] described a patient with PD given monthly intraventricular injections of GDNF for >1 year. No improvement in symptoms was observed. Adverse effects included nausea, loss of appetite, tingling, L'hermitte sign, intermittent hallucinations, depression, and inappropriate sexual conduct. There was no evidence of significant regeneration of nigrostriatal neurons or intraparenchymal diffusion of the ICV GDNF to relevant brain regions. On post-mortem study, there was no restoration of the dopaminergic cells. They concluded that alternative GDNF delivery systems should be explored.

An intraparenchymal route of administration may prove to be an effective way of delivering GDNF to the brain, and a recent phase I safety trial supports this view.[85] This study has reported that 1 year after long-term infusion of GDNF directly via a pump and catheter into the postero-dorsal putamen of five PD patients, significant improvements were seen clinically in off-medication motor symptoms and dyskinesias, and there was increased putamen dopamine storage on positron emission tomography scans. In addition, the side effects of the previous studies were not encountered, and there appeared to be no major neurosurgical complications with this approach. Thus, it appears that direct injection into the striatum can be achieved safely in PD patients and seems to be a much more effective form of GDNF delivery in humans.

In light of these promising results, a larger controlled trial of GDNF has commenced. Nutt et al.[240] described a double-blind, placebo-controlled, sequential cohort study for 8 months, comparing the effects of monthly ICV administration of placebo and 25, 75, 150, 300, and 500–4000μg of GDNF in 50 patients with PD. An open-label study extended exposure up to an additional 20 months and maximum single doses of up to 4000μg in 16 patients. Laboratory testing, adverse events, and Unified Parkinson's Disease Rating Scale (UPDRS) scoring were observed at 1- to 4-week intervals throughout the studies. Twelve patients received placebo and seven or eight patients were assigned to each of the other GDNF dose groups. 'On' and 'off' total and motor UPDRS scores were not improved by GDNF at any dose. Nausea, anorexia, and vomiting were common, hours to several days after injections of GDNF. Weight loss occurred in the majority of patients receiving ≥75μg doses of GDNF. Paresthesias, often described as electric shocks, were common in GDNF-treated patients, were not dose related, and resolved on discontinuation of GDNF. The open-label extension study had similar adverse events and lack of therapeutic efficacy. The investigators concluded[240] that GDNF administered by ICV injection remains biologically active, as evidenced by the spectrum of adverse events. However, it did not improve the parkinsonian state, possibly because it did not reach the target tissues in the striatum and SNpc. The lack of efficacy in this trial does not disprove the rationale for NTFs in PD but suggests that other means to target their delivery to the striatum and SNpc may be necessary to produce therapeutic effects and to reduce adverse effects. Direct intranigral or intrastriatal injection or deliveries of the GDNF gene to these targets are strategies worthy of further investigation.

The experimental data (animal and clinical studies) suggests that various members of the GDNF family, notably GDNF, might protect or rescue dopamine neurons from various insults. However, because of difficulties in reaching the target tissues in the brain, and the small numbers of patients tested so far, its clinical efficacy is yet to be demonstrated.

4.2 Aging and Mild Cognitive Impairment

4.2.1 Experimental Studies

In a unique study, Smith et al.[166] measured the number and volume of cholinergic immunolabeled neurons in the basal forebrain regions of Rhesus monkeys and found an age-related decline that was almost completely reversible by administration of human NGF. These authors suggested that subcortical structures are more vulnerable to aging effects than the cortex; however, no human studies have been reported.

4.3 Alzheimer Disease

4.3.1 Experimental Studies

NGF is the most widely examined neurotrophin in experimental models of AD. Several studies have found that ICV infusion of NGF prevents the retrograde degeneration of cholinergic neurons.[186,295] Hefti[186] assessed whether NGF was able to affect

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survival of central cholinergic neurons after axonal transections in adult rats. The septohippocampal pathway, transected unilaterally by cutting the fimbria, mimics the cholinergic deficit of the septohippocampal pathway loss in AD. Animals were implanted with a cannula through which NGF or control solutions were injected intraventricularly over 4 weeks. The lesions reduced the number of large cell bodies, as visualized by Nissl staining in the medial septal nucleus and in the vertical limb of the diagonal band of Broca. Furthermore, in the same nuclei, they reduced the number of cell bodies positively stained for acetylcholine esterase (AChE). On the lesioned sides, the number of cholinergic cells in medial septal nucleus and the vertical limb of the diagonal band was reduced by 50%, as compared with the number on contralateral sides. On lesioned sides of animals chronically treated for 4 weeks with NGF, the number of AChE-positive cells in these areas was reduced only by 12%, compared with control levels. These findings suggest that fimbrial transections resulted in retrograde degeneration of cholinergic septo-hippocampal neurons and that NGF treatment strongly attenuated this lesion-induced degeneration.

NGF ‘knockout’ mice showed deficits in spatial memory in the Morris water maze[296] that correlated with losses in the ChAT-positive basal forebrain neurons. In addition, the production of antibodies to NGF by transgenic mice resulted in similar developmental, behavioral and pathologic problems to those observed in NGF knockout mice and human AD.[297,298] Studies of the effects of intranasal administration of NGF or intraparenchymal injection of galantamine in these mice[299] using a variety of regimens at different ages (2, 6, and 6.5 months) showed complete restoration of the number of ChAT-immunopositive neurons to the normal range. In addition, NGF reversed the deposition of hyperphosphorylated tau, the major component of the paired helical fragments in AD, in the 2- and 6-month-old mice and markedly reduced the amyloid plaques in the 6- and 6.5-month-old mice. However, it had no effect on deposited amyloid precursor protein (APP) in cerebral vessels. Galantamine removed the amyloid plaques almost as effectively as NGF but in contrast with NGF, it also removed the APP deposits and had no effect on hyperphosphorylated tau. Surprisingly, the acetylcholinesterase inhibitors tacrine and physostigmine had no effect on any of these parameters. These studies indicate that lack of available NGF begins the process of plaque deposition and tangle formation.

Studies performed in aged rats demonstrate that NGF infusions can reverse the age-associated decline in basal forebrain cholinergic neurons and correct spatial memory deficits.[300] NGF can apparently also prevent basal forebrain cholinergic neuron degeneration in non-human primate brain.[301-303] Numerous studies have used rat models in which the connection between the septum and the hippocampus is disrupted by severing the fimbria-fornix. This prevents the retrograde transport of NGF down cholinergic fibers to the cell bodies. These studies found that the resulting cholinergic cell atrophy could be abolished by ICV administration of NGF.[184-188] This was also true with regard to the cognitive deficits arising from these lesions.[184,304,305] ICV infusion of NGF also improved age-related deficits on the Morris water maze test and cholinergic function in older rats.[300,306,307]

Other routes of administration are also feasible. Retroviral transfected fibroblasts expressing NGF successfully rescued cholinergic neurons after a fimbria-fornix lesion in rats,[308] and injections of NGF-expressing fibroblasts into the tissue adjacent to the basal forebrain restored the number of ChaT-positive subcortical neurons after 3 months in aged Rhesus monkeys with atrophy in the cholinergic basal forebrain.[166] In other studies, researchers injected I125-NGF into the olfactory bulb of rats. They found that the NGF was retrogradely transported specifically to basal forebrain cholinergic nuclei.[309] Similarly, radiolabeled NGF was transported into the brain following intranasal administration.[310,311]

In summary, of all the factors tested, NGF appears to be the most effective in improving the survival and maintenance of cholinergic neurons, and therefore may be a promising therapeutic agent for AD.

4.3.2 Clinical Studies

In two clinical trials conducted in the 1990s, Swedish patients with AD were treated with mouse NGF for up to 3 months. Some improvements were seen in a few cognitive tests, but the results were different in each patient, and there were several adverse effects, such as loss of weight and appetite, and significant back pain.[241,242] From these limited trials it could be concluded that ICV NGF administration may cause certain potentially beneficial effects. Working on the hypothesis that these adverse effects could be avoided by delivering the factor to precise cellular targets, a small clinical phase I trial of NGF gene therapy in AD was completed. The results were interpreted as being encouraging enough for larger, controlled trials of NGF gene delivery to commence.[243] A fundamental issue with neurotrophic approaches for the cholinergic system in AD is that the neuronal loss in this disease is widespread and that there are multiple neurotransmitter deficits.[312] Therefore, NGF therapy for AD seems unlikely to produce clinical benefits as striking as those described for GDNF factor infusion in PD,[85] where only a localized set of neurons

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needs protection/rescue. Alternative routes of administration, and/ or lower doses of NGF, perhaps combined with low doses of other NTFs, may shift this balance in favor of positive effects. Taken together, the results of these case studies indicate that NGF may counteract cholinergic deficits in AD, and suggest that further clinical trials of NGF infusion in AD are warranted.

4.4 Huntington Disease

4.4.1 Experimental Studies

The implantation of genetically engineered cells that release BDNF, NTF3, or NTF5 in the striatum of animals treated with the excitotoxin quinolinic acid was shown to promote the survival of striatal projection neurons. BDNF was the most effective survival agent,[313,314] and NTF3 was the most successful at initiating differentiation.[315] In addition, GDNF and BDNF were found to protect the majority of striatal projection neurons from excitotoxic lesions in the HD model.[316,317] To date, no human studies have been performed with NTFs in HD.

4.5 Amyotrophic Lateral Sclerosis

4.5.1 Experimental Studies

In a mouse mutant model of progressive motor neuropathy, adenovirus-mediated intramuscular gene transfer of NTF3 produced a 50% increase in life span, reduced the loss of motor axons, and improved neuromuscular function.[318]

The development of IGF-1 for the treatment of ALS is based on cell culture findings that chick motor neuron survival is promoted by IGF-1[319] and by the demonstration of stimulatory actions of IGF-1 and IGF-2 on motor neuron regeneration after sciatic nerve injury *in vivo* in rats and mice.[320-322] The mechanisms of action of IGFs in animal models of motor neuron injury remains unclear, and it is not known whether the biologic effects are caused by direct actions on motor neurons or, as suggested by some *in vitro* studies, are mediated by non-neuronal cells.[323] In support of the latter, IGF-1 expression is increased in astrocytes in the brain stem of adult rats after facial nerve transaction.[324] After rat sciatic nerve transection, IGF-1 was expressed by invading macrophages, whereas the IGF-1R localized to Schwann cells.[325]

4.5.2 Clinical Studies

An initial-phase I/II clinical trial, found that BDNF apparently increased survival rate and retarded the loss of pulmonary function in patients with ALS.[244] However, these findings were not replicated. Nevertheless, in a multicenter, randomized, double-blind, placebo-controlled parallel-group phase III study,[245] a total of 1135 patients with a diagnosis of probable or definite ALS, were randomized to receive either placebo (387 patients) or BDNF (748 patients), including 374 patients in each of two dose groups: 25 μg/kg/day or 100 μg/kg/day by injection. Inclusion criteria included percent predicted forced vital capacity ≥60%, age 21–80 years, ALS Functional Rating Scale score >18. It was found that survival in patients treated with 25 μg/kg BDNF was identical to placebo, but there was a trend toward increased survival in the 100 μg/kg group. The survival rate exceeded the initial expectations of the study.[245] The 9-month probability of survival was approximately 85% across all groups. This diminished the power of the study.[245] Among the 60% of patients with baseline forced vital capacity of ≤91%, survival was significantly greater for 100 μg/kg BDNF than placebo. Unpublished preclinical studies have indicated that an increased frequency of bowel motility or diarrhea is a pharmacologic action of BDNF.[288] 20% of patients treated with 100 μg/kg BDNF reported altered bowel function as an adverse effect in the first 2 weeks of administration. They were defined as BDNF ‘responders’, and had a significantly better 9-month survival period compared with placebo (97.5% vs 85%). However, the study failed to show the benefit of BDNF treatment for the primary endpoints; although the primary endpoint analysis failed to demonstrate a statistically significant survival effect of BDNF in ALS, *post hoc* analyses showed that those ALS patients with early respiratory impairment and those developing altered bowel function showed statistically significant benefit. In addition, *post hoc* subgroup analysis showed that patients undergoing BDNF treatment had a survival advantage over those at greater risk of dying during the time course of the study. This result may not indicate that ‘BDNF works only in sick patients’. More likely, this result highlights an issue that is best characterized as a ‘signal-to-noise’ problem. To detect a decrease in mortality in a clinical trial in ALS, the study population must contain enough at-risk individuals to detect a survival benefit, and the trial duration must be of sufficient length to accrue an adequate number of events (deaths). In this phase III study, the large proportion of patients who were likely to survive within the 9 months of the study period, lowered the possibility of detecting a survival effect of BDNF. Based on the *post hoc* analyses of the current study, there is a rationale to conduct future trials in patients with advancing respiratory decline. Dose regimens in future studies will attempt to deliver higher concentrations of BDNF to receptors on motor neurons, either by higher systemic doses or by intrathecal infusion.

Another study investigated the safety and tolerability of recombinant methionyl human BDNF infused intrathecally by means of an implanted pump in patients with ALS.[246] Twenty-five patients

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with probable or definite ALS were treated with either BDNF (25, 60, 150, 400, or 1000 μg/day) or placebo in a 12-week, randomized, double-blinded, sequential, dose-escalation study. Test treatment was interrupted by a washout period from days 11 to 25 to allow the evaluation of laboratory safety measures. In each dose cohort, four patients received BDNF and one received placebo. On completion of the double-blind period of the study, all patients continued receiving BDNF in an open-label extension for up to 60 weeks. Lumbar CSF samples were taken periodically from all patients to measure BDNF levels. Days after the initiation of infusion, the majority of patients receiving BDNF reported mild sensory symptoms, including paresthesias or a sense of warmth, which were usually confined to the lower limbs and were frequently exacerbated by neck flexion. In most instances, these symptoms decreased or even disappeared over several weeks. Sleep disturbance, dry mouth, agitation, and other behavioral effects were encountered at higher doses (>150 μg/day) and necessitated dose reductions. The spinal CSF levels of BDNF were directly related to dose, with a lumbar-to-cervical ratio of approximately 4 : 1. The investigators concluded[289] that intrathecal delivery of BDNF in doses of up to 150 μg/day was well tolerated and appears feasible. The reversible effects on the CNS with higher doses indicate that BDNF can be delivered cranially against CSF flow. The small number of patients and the design of the study did not permit conclusions to be drawn about the efficacy of the treatment.[246]

Clinical trials of subcutaneous treatment with human recombinant CNTF failed to ameliorate symptoms of ALS while producing adverse effects.[247-249] Patients were randomized, receiving 30 or 15 μg/kg CNTF or placebo subcutaneously three times a week for 9 months.[247] The most common dose-limiting toxic events reported by patients receiving CNTF were febrile reactions in some patients, fatigue, aphthous stomatitis, anorexia, weight loss, asthenia, nausea, injection site reactions, and cough. Their incidence was greatest in the first 2 months of the study, although a significantly greater incidence of anorexia, asthenia, and cough persisted in the CNTF groups through the end of the 9-month treatment period. Headache, increased salivation, pain, and dyspnea were reported with equal frequency in all treatment groups. A decrease in both mean supine and standing blood pressure was noted in the CNTF-treated patients without evidence of orthostatic change, indicating that these patients were not volume-depleted secondary to poor oral intake or vomiting, or both. Two unexplained adverse effects of CNTF were the cough experienced by most of the treated patients and the dose-related frequency of aphthous stomatitis. This cough was unaccompanied by radiographic evidence of pulmonary inflammation or consolidation and was unresponsive to antitussives, inhaled anticholinergics, and bronchodilators.

In another single-center, randomized, single-blind (to the patient) study of subcutaneous injection of placebo or CNTF, the toxicity of both single and multiple subcutaneous injections of CNTF in 72 patients with ALS, in doses ranging from 2 to 100 μg/kg were examined.[248] Adverse events were generally dose related and ranged from mild to severe. The tolerability of daily subcutaneous CNTF was equivalent to placebo at doses ≤5 μg /kg/day. At higher doses, anorexia, weight loss, reactivation of herpes simplex virus (HSV1), labialis/stomatitis, cough, and increased oral secretions occurred. In most cases, weight stabilized after stopping CNTF and returned to baseline over the next 4–10 weeks. Cough or increased oral secretions generally resolved within 48 hours of stopping the drug. The mechanisms of both cough and anorexia are unknown.

However, one study showed that intrathecal delivery of CNTF was not associated with the side effects observed with systemic delivery, but the authors did not provide data on efficacy.[250]

Recombinant human IGF-1 (rhIGF-1) is a naturally occurring peptide with multi-target neurotrophic potential on motor neurons as well as the neuromuscular junction and muscle.[326] rhIGF-1 has promoted the survival of spinal and facial motor neurons in experimental models of peripheral nerve transection and excitatory amino acid induced cell death[327,328] and has also promoted peripheral nerve regeneration in motor nerve axotomy models.[329] Receptors for rhIGF-1 have been reported to be up-regulated in the spinal cord of patients who have died of ALS.[117,330] Moreover, a clinical survey indicates that important alterations in the peripheral IGF-1 and IGFBP systems are observed in ALS patients.[331] Significant increases were found in three of four of the main circulating IGFBPs in ALS patients, whereas serum IGF-1 was significantly reduced. These studies provided the background for randomized placebo controlled trials of rhIGF-1 in ALS.

Two randomized controlled trials of rhIGF-1 in ALS have so far been published in a definitive form. The larger trial[251] showed the slowing of progression of functional impairment and quality of life, but this was not found in the second, slightly smaller trial.[252] The two studies comprised 300 patients treated with rhIGF-1 and 149 given placebo. The North American randomized trial[251] compared 176 patients receiving rhIGF-1 0.05–0.1 mg/kg/day with 90 controls (placebo), the European trial[252] compared a dose of rhIGF-1 0.1 mg/kg/day in 124 patients with 59 controls (placebo). Change in disease progression as determined by the Appel ALS Rating Scale total score at a dose of 0.1 mg/kg/day of

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rhIGF-1 subcutaneously after 9 months' treatment. The level of significance was lower in the European trial (weighted mean difference −3.30, 95% CI −8.68, 2.08) than in the North American trial (weighted mean difference −6.00, 95% CI −10.99, −1.01). Evaluation of adverse events showed an increased risk of injection site reactions/inflammation with rhIGF-1. The drug was otherwise safe and well tolerated. Recombinant human IGF-1 may be modestly effective but the evidence currently available is insufficient for a definitive assessment. Further studies are needed to determine whether NTFs might be effective in the treatment of ALS.

5. Pharmacogenomics of NTFs

Pharmacogenomics is a new field of research that examines how polymorphisms in specific genes affect an individual's risk of disease and the response to drugs. Several such studies have been performed to investigate the association between NTF polymorphisms and the risk of neurodegenerative disorders.

5.1 Parkinson Disease

One study examined 20 single nucleotide polymorphisms in 18 candidate genes, for association with PD and found that homozygosity for the Val66Met polymorphism of the BDNF gene occurs more frequently in patients with PD than in unaffected controls.[332] However, another recent study did not find any association between BDNF Val66Met polymorphism and PD in a Swedish population.[333]

Only one association study of GDNF in PD has been performed so far. It identified a novel variant of GDNF, a potent survival factor for nigrostriatal dopaminergic neurons, in 1 of 30 patients with PD. However, the alteration did not change the predicted amino acid sequence, and it was also found in 1 of 20 patients without PD.[181] Further studies and meta-analyses are needed before we can draw conclusions concerning the effect of NTFs, and especially genetic variations in BDNF, on the risk of PD.

5.2 Alzheimer Disease

Of the three association studies of BDNF and AD, one showed that a single nucleotide polymorphism (−270C/T) within the BDNF gene is associated with late-onset disease in a Japanese population.[334] The second study found an association between the BDNF 196A/G polymorphism and sporadic AD,[335] and the third, performed in a German population, found that the BDNF −270C/T polymorphism is a relevant risk factor for AD, particularly in patients lacking the ApoE epsilon 4 allele.[336]

It is of interest that a missense mutation (Gly63Glu) of the NTF3 gene was found to be associated with AD in a Japanese population.[105]

5.3 Amyotrophic Lateral Sclerosis

Three association studies examined the role of CNTF in ALS. One found genetic variation in the CNTF receptor-α gene in patients with familial ALS.[337] The second reported that CNTF acts as a modifier gene, leading to early onset of disease in patients with FALS and SOD-1 mutations, in patients with sporadic ALS, and in the hSOD-1G93A mouse model.[105,338] The third study found a decrease in CNTF expression in the lateral corticospinal tract of the spinal cord in patients with ALS. This might be a feature of ALS, and might be related to motor neuron loss.[339]

More studies are needed to determine whether polymorphisms in NTFs or their receptors influence the risk of developing neurodegenerative disorders.

6. Delivery of NTFs Into the Brain

One of the significant challenges in the potential clinical use of NTFs is their delivery into the CNS. NTFs are hydrophilic, typically basic, monomeric or dimeric proteins, mostly in the size range of 5–30 kDa. They have low oral bioavailability and are restricted by the blood-brain barrier from entering the CNS following systemic administration.[340] In addition, most of the NTFs have short half-lives, poor pharmacokinetic profiles, rapid enzymatic inactivation, multiple clearance processes, and potential immunogenicity and sequestration. These characteristics restrict their access to the CNS by binding proteins and other components of the blood and peripheral tissues. Therefore, clinical effectiveness of NTFs in disease affecting the CNS will probably be determined by the ability of researchers to develop a strategy of targeted delivery. Achieving significant CNS target site concentrations while limiting systemic exposure and distribution to peripheral sites of action will lessen unwanted pleiotropic effects and toxicity. Best targeting is obtained with local introduction of NTF intraparenchymally, by direct injection/infusion or by implantation of delivery vectors such as polymer matrices or genetically modified cells. However, this method is limited by diffusion restrictions and invasiveness. ICV/intrathecal administration is less invasive and allows access to a much wider area of the CNS through CSF circulation pathways. However, the diffusional and cellular barriers to penetration into surrounding CNS tissue and the significant clearance of CSF into the venous and lymphatic circulation are limiting. Unconventional delivery strategies such

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as intranasal administration may offer some degree of CNS targeting with minimal invasiveness.

However, these methods have relatively limited neurotrophic delivery in terms of the extent of their diffusion within the CNS, and also generate a range of practical and safety issues that arise through the need to be able to chronically deliver NTFs. In addition, it would clearly be advantageous for neurodegenerative patients to receive a single 'one-off' injection of NTFs. As a result, more efficient delivery systems, including the use of viral vectors, have been sought. There are many different types of viral vectors that can be considered for delivering NTFs, including adenovirus, adeno-associated viral vectors, and lentiviruses. Each of these approaches has their own particular merits as well as disadvantages.[341] An approach using a viral vector delivery system generates issues of safety, in that if problems arise, it is easier to switch off an infusion than a virally delivered trophic factor. Nevertheless, the ease and efficiency of delivery of viral vectors have clearly been shown for lentiviruses and adeno-associated viruses,[342] and thus they remain possible therapeutic options for the future in patients with PD. Clearly, more experiments in nonhuman primate models will be necessary to prove that this technique leads to long-term NTF expression, as well as proving that the procedure is completely safe. In this respect, the use of a regulatable promoter to provide a means of controlling expression of the transgene may prove necessary.

Recently, the use of drug-releasing (NGF or GDNF) biodegradable microspheres implanted into brain has been investigated as an alternative strategy to NTF administration.[343-347] Microspheres can be implanted by stereotaxy, are biocompatible and totally biodegraded in the brain and can release bioactive NTFs for at least 2 months. Jollivet et al.[347] demonstrated a long-term effect of intra-striatal GDNF-releasing microspheres in a rat model of PD. The functional recovery of the animals observed after the treatment was accompanied by an increase in tyrosine hydroxylase-immunoreactive fiber density in the striatum, visible 32 weeks after implantation, when microspheres were completely degraded. This confirms the possibility of delivering GDNF and other NTFs through implantable biodegradable microspheres. Moreover, the restorative effect obtained persists after complete degradation of these microspheres. This point is important for future applications of this strategy in the treatment of PD.

7. Future Directions

NTFs have been used in many experimental studies and several phase I/II and phase III clinical trials for the treatment of neurodegenerative disorders. Most of the data imply that NTFs are promising candidates in the treatment of these patients. However, their poor pharmacokinetic profiles and the difficulties associated with their delivery into the brain reduce their clinical potential. Therefore, more research is needed to study the molecular mechanism by which NTFs work in both normal and pathologic conditions. This, together with development of novel drug delivery systems into the brain, will facilitate their access to the required site within the brain and minimize their adverse effects. Further studies in larger, placebo-controlled multicenter trials are warranted before NTFs can become part of the therapeutic armamentarium of neurodegenerative diseases.

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Correspondence and offprints: Dr *Daniel Offen*, Felsenstein Medical Research Center, Beilinson Campus, Petah Tiqwa 49100, Israel.
E-mail: doffen@post.tau.ac.il

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