

Glial Cell Line-Derived Neurotrophic Factor (GDNF): A Comprehensive Review

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Introduction

Glial cell line-derived neurotrophic factor (GDNF) was originally isolated based on its ability to promote the survival and differentiation of dopaminergic (DA) neurons in primary cultures of embryonic ventral midbrain (1,2) (see Figure 1). Protein purification, sequencing and cloning revealed it to be a distantly related member of the transforming growth factor β (TGF β) superfamily. Like other members of this superfamily, GDNF is synthesized as a precursor that is processed into a mature disulfide-bonded dimer. The effects of GDNF on DA neurons in midbrain cultures are potent and relatively specific, as GDNF selectively acts on DA neurons with an EC₅₀ of approximately 40pg/ml (increasing DA cell number, dopamine uptake, cell size and neurite length) without affecting non-DA neurons or glia, which are also present in these cultures (no effects on GABA and serotonin uptake, overall number of neurons, astrocytes or oligodendrocytes). GDNF also promotes survival and regrowth of cultured DA neurons damaged by the neurotoxin 1-methyl-4-phenylpyridinium (MPP⁺)

(3). These *in vitro* findings suggest that GDNF could be a useful therapy for Parkinson's disease (PD), which results from degeneration of midbrain nigral DA neurons that innervate the striatum.

A steady stream of positive effects of GDNF in animal studies has sustained this molecule's therapeutic potential for PD. Also, it was demonstrated that GDNF expression is not limited to the nigrostriatal system and that GDNF can affect other populations of neurons, most notably motoneurons on which GDNF has the same pronounced potency as seen in DA neurons. Surprisingly, GDNF mRNA is expressed in many peripheral tissues at levels higher than that in most brain regions, suggesting possible additional roles for GDNF outside the nervous system. Some of those roles were revealed in recent GDNF-null mice studies. Each of the aforementioned issues is briefly reviewed in this article.

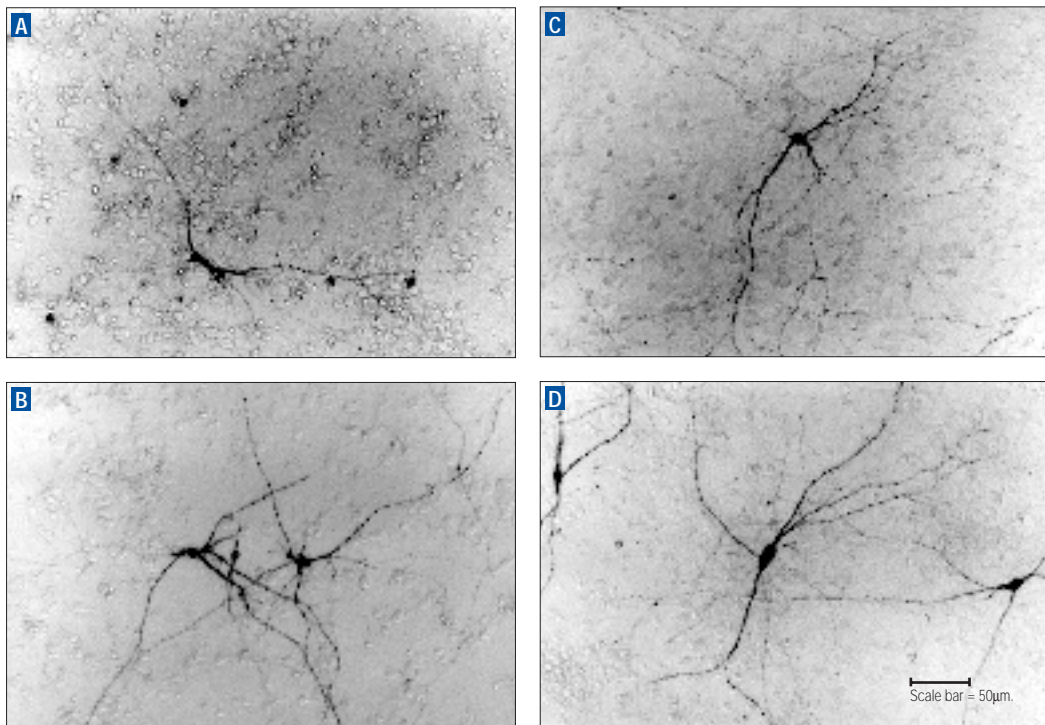
GDNF in Animal Models of Parkinson's Disease

Three lines of evidence from the laboratories of Hoffer and Olson (4-6) suggest that GDNF can act *in vivo* on nigral DA neurons. First, GDNF elicits a dose-dependent increase in the growth of nigral grafts *in oculo*, which is associated with increased survival and fiber outgrowth of tyrosine hydroxylase-immunoreactive (TH-IR) DA neurons within the graft (4). Second, intranigral-injected GDNF elicits sprouting of TH-IR neurons and increased DA turnover in the adult rat nigrostriatal system (5), suggesting that functional GDNF receptors exist in the

Figure 1. ►

Morphology of TH-immunoreactive dopaminergic neurons before and after treatment with GDNF.

Dissociated midbrain cultures were incubated for 12 days without (Panels A and C) or with (Panels B and D) 3pM of purified GDNF and stained for tyrosine hydroxylase (TH). Cultures were maintained in defined (Panels A and B) or serum-containing (Panels C and D) medium. The GDNF concentration was calculated based on an average molecular mass of 39kDa. Conditions for the collection, maintenance and treatment of neurons was as described in reference 2.



adult. Third, intrastriatal-injected GDNF can be transported retrogradely, along the axon, back to nigral TH-positive cell bodies and dendrites (6), as would be expected of a target-derived trophic factor for DA neurons *in vivo*.

6-hydroxydopamine (6-OHDA) Rat Model: In Hoffer's laboratory (7,8), a nigral cell body lesion was produced by injecting 6-OHDA unilaterally into the rat medial forebrain bundle. Four weeks later, a single intranigral injection of GDNF produced a significant reduction in lesion-induced rotational behavior and restored the dopamine content and TH-IR neuron phenotype in the lesioned substantia nigra over a five week period. In Gash's laboratory (9), rats were given a single intranigral injection of GDNF. Twenty-four hours later, the animals received 6-OHDA infusion into either the ipsilateral substantia or striatum. Animals were euthanized two weeks later for TH-IR immunohistochemical analyses. It was shown that GDNF protected mature midbrain DA neurons from the neurotoxic effect of 6-OHDA *in vivo*. In Björklund's laboratory (10), rats received unilaterally 6-OHDA infusions in the striatum. GDNF, injected intranigral every other day (starting on the day of lesion) over a four-week period, completely prevented nigral cell death and atrophy. Interestingly, this model resulted in a delayed and progressive nigral cell death more closely related to that seen in human PD as demonstrated with fluorescent retrograde tracer injections and TH immunocytochemistry (10).

Axotomy Rat Model: Nigral neurons were induced to degenerate by transecting their axons within the medial forebrain bundle. Daily intranigral injection of GDNF for two weeks significantly prevented the loss of nigral TH-IR neurons resulting from the transection (11).

MPTP Mouse Model: The DA neuron neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces PD-like symptoms in man (12). Intrastriatal or intranigral injection of GDNF before MPTP administration protected the dopamine system, as shown by TH-IR cell bodies and fiber densities, and levels of dopamine and its metabolites (13). In another experiment, GDNF injected after MPTP substantially restored dopamine levels and TH-IR fiber densities. Of importance, in both cases, motor performance also improved (13).

MPTP Non-Human Primate Model: Significantly, this model closely mimics the clinical symptoms found in PD, as monkeys are more closely related to the human, than rodents are, in brain organization and function. In Gash's laboratory (14), monkeys were made PD-like by MPTP infusion into the right carotid artery. Three months after MPTP administration, GDNF was injected once every four weeks by one of three routes: intranigral, intracaudate or intracerebroventricular (ICV). GDNF recipients showed significant functional improvements in

bradykinesia, rigidity and postural instability with all three routes of administration. On the lesioned side, GDNF increased nigral TH-IR fiber densities and dopamine levels in the midbrain and globus pallidus. This encouraging finding qualifies GDNF as the most interesting candidate factor to date for treating PD.

GDNF Effects on Motoneurons

In recent work (15-17), GDNF was demonstrated to be an extremely potent survival factor for motoneurons *in vitro*, supporting the survival of purified embryonic rat motoneurons in culture with an EC₅₀ of 0.1pg/ml (15). In culture, GDNF increases motoneuron cell number and neurite outgrowth, and choline acetyltransferase (ChAT) activity (18).

In vivo, GDNF almost completely rescues the motoneurons from cell death following axotomy of facial nerve in newborn rats (15,16,19) and of sciatic nerve in neonatal mice (17). Furthermore, Oppenheim *et al.* (17) showed that naturally occurring, as well as axotomy-induced, cell death in developing avian motoneurons can be prevented by GDNF. Uniquely, GDNF also completely prevents lesion-induced motoneuron atrophy in neonatal rodents (15,17,18). This has not been observed with other identified motoneuron trophic factors.

Evidence that GDNF mRNA expression is regulated temporally and spatially in skeletal muscle (15) and that the protein is transported retrogradely by spinal motoneurons (19) in neonatal rats suggests GDNF may serve physiologically as a target-derived trophic factor for motoneurons.

In addition to developing motoneurons, injured adult motoneurons also can respond to GDNF. Following axotomy, adult motor neurons can survive, but with decreased ChAT immunoreactivity. GDNF prevents such axotomy-induced decrease of ChAT immunoreactivity in the facial nucleus of adult rats (19). In a different type of lesion, ventral root avulsion which induces adult motoneuron death, GDNF rescues injured spinal motoneurons and induces hypertrophy following avulsion in adult mice (20) and rats (21). Therefore, GDNF may be useful in the treatment of motoneuron disorders like amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy.

Although quite promising for the treatment of PD, the potential for GDNF in treating motoneuron diseases may be limited. In the progressive motor neuronopathy (PMN) mouse mutant (22), in contrast to CNTF (23), GDNF does not prevent axonal degeneration or disease progression. However, GDNF does rescue motoneuron cell body survival. GDNF's therapeutic usefulness may be restricted to cotreatment with other factor(s) such as CNTF which acts on the nerve process (22).

GDNF mRNA Distribution

Information on GDNF mRNA expression in developing rat brain supports a role of GDNF as a dopaminotrophic factor and the notion that GDNF may affect other CNS neurons, as it also is expressed in other brain regions. GDNF has been detected by *in situ* hybridization and by reverse transcription PCR (RT-PCR) in the nigrostriatal system, including embryonic and neonatal striatum, embryonic ventral midbrain and substantia nigra Type I astrocytes (4,24,25). However, low levels of widespread GDNF mRNA expression were observed in non-DA areas in newborn rats, including the cerebellum, spinal cord, thalamus, cortex and basal forebrain (4,24). While GDNF mRNA was undetectable in normal adult rat central nervous system (CNS) by *in situ* hybridization, it was upregulated in seizure-induced striatum, hippocampus and neocortex (26,27), suggesting a possible role for GDNF in response to injury. A more sensitive RT-PCR assay demonstrated expression in normal adult rat and human striatum, hippocampus, cortex, spinal cord and cerebellum (28,29). Additionally, when the brain is dissected and RNA is isolated from different areas, GDNF mRNA can be detected by RT-PCR in almost every brain region studied in all ages ranging from embryonic day 11.5 (E11.5) to adult (30). In the rat brain, postnatal day 10 (P10) striatum and embryonic spinal cord shows the maximal expression (31).

Non-CNS expression of GDNF mRNA has been studied by RT-PCR (30,32), ribonuclease protection assay (RPA) (31) and *in situ* hybridization (33). The strong expression of GDNF mRNA in the kidney, intestine and stomach of the early rat embryo has been verified by all methods (30-33). GDNF transcripts are also present in muscle, cartilage, testis, lung and blood. The presence of GDNF mRNA within cartilage development links GDNF to other TGF β superfamily members, most notably to bone morphogenetic proteins (34). While RT-PCR and RPA analyses of mRNA expression are sensitive, only *in situ* hybridization can reveal the cell types expressing the mRNA within a tissue. From the expression pattern revealed by *in situ* hybridization, Suvanto *et al.* (33) predicted important roles for GDNF in the early differentiation of the kidney tubules and the innervation of the gastrointestinal tract. Those predictions find strong support in the most recent observations from mice carrying targeted null mutations in the GDNF gene discussed below.

All RT-PCR and RPA assays (28-32,35) demonstrate that GDNF mRNA is expressed in two forms, presumably by alternative splicing, in most tissues and Schwann cells. Sequence analyses revealed that one transcript contains a 78bp deletion corresponding to a loss of 26 amino acids and a Gly²⁵→Ala²⁵ substitution within the prepro region of the coding sequence. However, identical

mature GDNF proteins are produced from the two mRNAs. The significance of the shorter splice variant currently is unknown. It could conceivably influence the processing of the GDNF precursor (31). Recently, an additional transcript has been identified (29) which encodes a protein that lacks a prepro region and the consensus signal peptide, and differs in its first 18 amino acids from the predicted mature GDNF.

GDNF Effects on Other Neuronal Populations

In vitro: Dreyfus and Black's group demonstrated (36) that GDNF is the most potent survival and differentiation factor yet described for cultured Purkinje cells, the efferent neurons of cerebellar cortex. Purkinje cells constitute a small subpopulation of the cells in dissociated cultures of rat cerebellar cortex. GDNF selectively enhances Purkinje cell survival and morphologic maturation with a maximal response at 1pg/ml. GDNF did not alter the overall number and morphology of neurons or glial cells also present in the same culture. As GDNF is a survival and differentiation factor for midbrain DA neurons and cholinergic motoneurons, collectively these findings suggest that GDNF might be a critical trophic factor at multiple loci within neuronal circuits that control motor function (36).

An initial study of GDNF's effects on neurons of the peripheral nervous system (PNS) revealed that GDNF promotes the survival and neurite outgrowth of cultured sympathetic neurons, parasympathetic ciliary ganglion neurons and sensory dorsal root ganglion neurons dissected from chick embryo (31,37,38). GDNF also modifies the phenotype of cultured sympathetic neurons by increasing the expression of vasoactive intestinal peptide and preprotachykinin mRNA (31). However, the potency of GDNF acting on these PNS neurons ($EC_{50} = 9.325\text{ng/ml}$) is much lower than that of GDNF acting on midbrain DA neurons ($EC_{50} = 40\text{pg/ml}$) (37). Comprehensive studies (39) of a wide variety of chick embryo PNS neurons (including sympathetic, parasympathetic proprioceptive, enteroceptive and cutaneous sensory neurons), showed that they become either less sensitive or more sensitive to GDNF as development proceeds. GDNF mRNA is expressed in all of the tissues innervated by the neurons that respond to GDNF (31, 38,39). This finding strengthens the physiological relevance of the *in vitro* observations.

In vivo: To assess GDNF's neurotrophic activity on adult locus coeruleus (LC) noradrenergic neurons, Arenas *et al.* (40) unilaterally implanted genetically-engineered fibroblasts expressing GDNF posterolateral to the rat LC. One day later, one group was selectively lesioned via an ipsilateral 6-OHDA injection rostral to the LC. Seven days after surgery, brains were processed for TH immunohistochemistry. In nonlesioned normal

LC, GDNF administration induced a mild sprouting and a 70% increase in the cytoplasmic soma of LC neurons. In lesioned LC, GDNF treatment resulted in a two and one-half-fold increase in TH levels, and hypertrophy and sprouting of LC neurons (40). It is possible that LC noradrenergic neurons may respond to the lesion by increasing GDNF receptor expression. Regardless of the mechanism by which 6-OHDA induces GDNF responsiveness in the LC, the induction of TH, hypertrophy and sprouting of noradrenergic nerve terminals after GDNF administration suggest an increased functional capacity of these cells. Moreover, it suggests GDNF may prevent and compensate for the degeneration of central noradrenergic neurons observed in Alzheimer's, Parkinson's and Huntington's diseases (40).

GDNF exerts protective effects on a variety of neurons and tissues. GDNF is protective to hippocampal, thalamic and amygdaloid neurons. Using a rat model of temporal lobe epilepsy, Martin *et al.* (41) showed that GDNF prevented kainate-induced seizures and seizure-associated neuronal cell loss of hippocampus (CA/pyramid cells), thalamus and amygdala, suggesting that GDNF may play a role in acute neural disorders

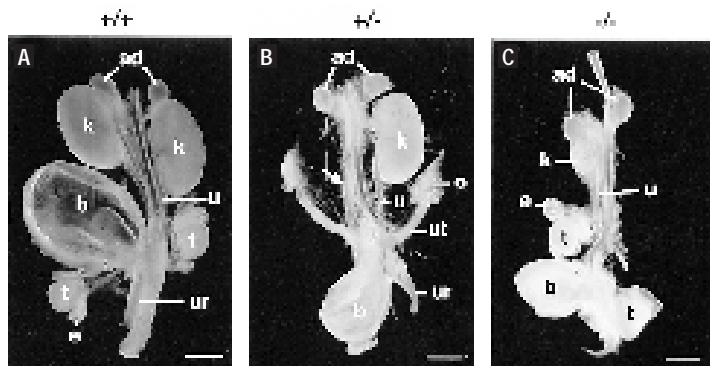
associated with excitotoxic processes. Oppenheim *et al.* (16) demonstrated that GDNF can protect embryonic chick interneurons of the isthmo-optic nucleus from programmed cell death. GDNF treatment allows successful transplantation of more mature spinal cord tissue. *In oculo*, GDNF increases survival and growth of postnatal spinal cord tissue transplants that would otherwise show minimal or no survival (42). GDNF attenuates a subset of behavioral deficits in aged rodents (43). Following implantation of polymer encapsulated GDNF-producing fibroblasts into the striatum of aged rats, the animals demonstrated increased activity and performed significantly better in bar pressing – an operant task. These results indicate that GDNF may be useful for treating age-related motor dysfunction resulting from striatal DA hypofunction (43).

GDNF-Null Mice

Recent studies on GDNF-deficient mice from the laboratories of Rosenthal, Barbacid and Westphal indicate that GDNF is essential for the development of the kidney and the enteric nervous system (44-46). GDNF-null mice fail to develop kidneys and to innervate the gastrointestinal tract, and die shortly after birth. This is not entirely surprising because, during embryogenesis, GDNF is expressed in the kidney and gastrointestinal tract as well as in the nervous system (30-33). What is surprising is that the development of DA and spinal motoneurons appears normal in the mutant mice (44-46). The mutant mice have deficits in certain peripheral neurons (dorsal root ganglion, sympathetic and nodose neurons), but not in dopaminergic, noradrenergic or cholinergic motor neurons (44). However, these studies do not rule out the distinct possibility that, in the adult, GDNF is a physiological survival factor for these CNS neurons. This issue cannot be addressed in the mutant mice because of their premature death.

Conclusion

GDNF was initially purified and cloned from a glial cell line in the search for a neurotrophic factor for midbrain DA neurons that degenerate in PD. Recent studies have extended the spectrum of GDNF bioactivities to a variety of central and peripheral neurons as well as to the kidney and gut. In the CNS, GDNF has potent trophic effects on midbrain DA neurons, facial and spinal motoneurons, LC noradrenergic neurons and cerebellar Purkinje cells. GDNF also has protective effects on hippocampal, thalamic and amygdaloid neurons. In the periphery, GDNF acts on sympathetic and parasympathetic




▲ Figure 2.

Analysis of urogenital system defects in GDNF newborn mutant mice. Panel A: Whole-mount of a dissected urogenital system from a normal newborn male mouse. Panel B: Right renal agenesis in a heterozygous GDNF^{+/-} female; the affected side reveals a blind-ending ureter without renal tissue at the tip (arrow). Panel C: Renal agenesis and severe dysgenesis observed in a GDNF^{-/-} male. A rudimentary kidney with its ipsilateral ureter is present; the contralateral side shows neither kidney nor ureter. Different combinations of agenesis/dysgenesis are observed in mice with mutant genotypes. Adrenal glands, gonads and the rest of the urogenital system components appeared macroscopically normal. Abbreviations: ad, adrenal gland; b, bladder; e, epididymis; k, kidney; o, ovary; t, testes; u, ureter; ur, urethra; ut, uterus. Scale bars = 1mm. Details of this experiment are described in reference 46.

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autonomic as well as various subpopulations of sensory neurons. During development, GDNF is essential for kidney formation and proper innervation of the gastrointestinal tract.

By virtue of its effects in cell culture and efficacy in animal studies, GDNF constitutes a powerful therapeutic candidate for several neurodegenerative diseases, especially PD and ALS. (Indeed, Amgen recently announced the first clinical trials of GDNF for PD.) However, attempts to treat a particular clinical deficit with GDNF may result in side effects in that GDNF appears to have multiple targets. This is highlighted by the adverse side effects of weight loss and hyperalgesia found in clinical trials of CNTF for ALS (47,48) and NGF for Alzheimer's disease (49) despite the efficacy of these factors in animal studies. Such side effects may, however, be overcome by the use of localized delivery and/or combinations of candidate factors that have synergistic effects at dose levels that do not produce significant side effects (50). One example of an improved method of delivery was recently published in *Nature Medicine*; using intrathecal delivery of CNTF for ALS, Aebischer *et al.* (51) were able to eliminate the limiting side effects observed with systemic delivery of CNTF.

The most recent characterization of the GDNF receptor complex (52-55) will certainly help to elucidate the mechanism of GDNF action, and lead to better strategies for use of GDNF to treat neurodegenerative diseases. While the jury is still out regarding any therapeutic potential of GDNF in clinical settings, one thing is certain, the tale of GDNF has only just begun. 

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rhGDNF	5µg	G2781

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