REVIEW

GDNF and protection of adult central catecholaminergic neurons

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Abstract

Neurotrophic factors are small proteins necessary for neuron survival and maintenance of phenotype. They are considered as promising therapeutic tools for neurodegenerative diseases. The glial cell line-derived neurotrophic factor (GDNF) protects catecholaminergic cells from toxic insults; thus, its potential therapeutic applicability in Parkinson's disease has been intensely investigated. In recent years, there have been major advances in the analysis of GDNF signaling pathways in peripheral neurons and embryonic dopamine mesencephalic cells. However, the actual physiological role of GDNF in maintaining catecholaminergic central neurons during adulthood is only starting to be unraveled, and the mechanisms whereby GDNF protects central brain neurons are poorly known. In this study, we review the current knowledge of GDNF expression, signaling, and function in adult brain, with special emphasis on the genetic animal models with deficiency in the GDNF-dependent pathways.

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Introduction

Neurotrophic factors (NTFs) are small natural proteins necessary for the development and survival of nerve cells as well as for the maintenance of their morphological and functional phenotype. The ‘neurotrophic hypothesis’ enunciates that the establishment and maintenance of neuronal networks require the release at the target structures of NTFs, which are uptaken by the nerve terminals and retrogradely transported to the soma of the projecting neurons (Ibanez 2007). Upon reaching the nucleus, NTFs induce a gene program that promotes neuronal survival and phenotype specification. Although the existence of ‘chemotactic’ influences between the growth cones of axons and their targets was already postulated by Cajal’s group (Cajal 1928), the modern concept of neurotrophism is based on the work of Hamburger & Levi-Montalcini (1949) who reported that the switch from cell survival to death observed during brain development depended on substances produced at the site where neurons were projecting, hence removal of a prospective target dramatically increased neuronal loss.

During the last decades, several proteins have been classified as NTF because of their effect on neuronal survival, differentiation (including synaptogenesis and neurite branching in vitro), maturation of electrophysiological properties, and plasticity. Due to their functional roles, NTFs are considered a promising tool to treat neurodegenerative diseases (Kirik et al. 2004), and particularly Parkinson’s disease (PD), a neurodegenerative disorder characterized by motor symptoms (tremor, bradykinesia, rigidity, and alteration of gait) (Lang & Lozano 1998, Fahn 2003) which affects over one million Europeans (de Rijk et al. 1997, de Lau & Breteler 2006). The etiology and pathogenesis of PD are essentially unknown, although several causative mechanisms, including alterations of protein folding/degradation, mitochondrial dysfunction and oxidative stress, neuroinflammation, and Ca2+ excitotoxicity have been proposed (Dauer & Przedborski 2003, Farrer 2006). PD is caused by the progressive loss of specific sets of neurons both in the brainstem and in the peripheral nervous system. From a clinical standpoint, the most critical neuronal population affected corresponds to the midbrain dopaminergic (DA) neurons in...
the substantia nigra (SN) pars compacta projecting to the striatum (nigrostriatal neurons), thus leading to dysfunction of the neuronal circuits in the basal ganglia and alteration of motor control. DA neurons in the neighboring ventral tegmental area (VTA) are less affected than SN neurons. Although the loss of nigrostriatal DA neurons is the most apparent pathological hallmark of PD, other cell types are affected even before SN cell death. Among those are the noradrenergic neurons in the locus coeruleus (LC) and cells in the dorsal nucleus of the vagus or in the sympathetic ganglia. Peripheral sympathetic denervation (loss of cardiac or celiac fibers) has been proposed to be an early marker for PD (Braak et al. 2003).

Among the various NTFs studied, the glial cell line-derived neurotrophic factor (GDNF) is the one more closely associated with PD, due to its potent trophic action on cultured DA neurons (Lin et al. 1993). GDNF is produced by striatal neurons and is necessary for maintenance of adult nigrostriatal DA neurons and other central and peripheral nuclei affected in PD (Pascual et al. 2008). In addition, administration of exogenous GDNF can prevent neurotoxic damage of midbrain DA neurons (Tomac et al. 1995a,b) and noradrenergic neurons in the LC (Arenas et al. 1995) as well. Hence, stimulation of endogenous GDNF production and/or administration of exogenous GDNF are considered therapeutic strategies of potential applicability in PD (Gill et al. 2003, Vastag 2010).

In this review, we briefly summarize the current knowledge on the role of GDNF in dopamine/catecholamine neuron maintenance during adulthood, emphasizing the data obtained from animal models of NTF deficiency.

**GDNF signaling in central catecholaminergic neurons**

GDNF is a distantly related member of the transforming growth factor-β (TGF-β) superfamily (Lin et al. 1993). Three GDNF homologues have been described so far, which include neurturin, persephin, and artemin. GDNF has raised special attention due to its potent effect on DA and noradrenergic neuron survival (Arenas et al. 1995, Gash et al. 1996; for a comprehensive review, see Kirik et al. 2004). The members of the GDNF family of trophic factors are often grouped as ‘dopaminotrophic’ factors. GDNF is expressed in several regions of the adult rodent brain, particularly in the striatum, anteroventral and anteromedial nuclei of the thalamus, and septum (Trupp et al. 1997, Pascual et al. 2008; Fig. 1), brain areas that receive prominent catecholaminergic afferent innervation (Lindvall & Stenevi 1978, Bjorklund & Hokfelt 1984). The identity of the striatal neurons expressing GDNF is not completely defined. Striatal cholinergic interneurons produce GDNF, but other unidentified neuronal types also contribute to GDNF production in this nucleus (Bizon et al. 1999). The site and mechanisms of GDNF release as well as the signals regulating GDNF production are also basically unknown. Stimulation of GDNF expression levels by neurotransmitters (mainly dopamine and adenosine) and inflammatory signals and the underlying mechanisms have been investigated (for a review, see Saavedra et al. 2008). A recent in vitro study has revealed the secretory pathways used by specific GDNF isoforms and the modulation of its release by KCl-induced depolarization (Lonka-Nevalaita et al. 2010). In addition, the proteases that

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**Figure 1** Schematic representation of regions with high GDNF expression levels in adult brains. Light blue represents the areas where GDNF mRNA or activity of the GDNF promoter is detected. CPu, caudate putamen (striatum); Acb, accumbens nucleus; Ms, medial septal nucleus; AVVL, anteroventral thalamic nucleus, ventrolateral part; AM, anteromedial thalamic nucleus. Ventricles are shown in gray.
seem to be involved in GDNF maturation have been described, which is different from the processing pathway of NGF and BDNF (Lonka-Nevalaita et al. 2010).

As other NTFs, GDNF is uptaken by axon terminals of projecting neurons and transported to cell soma (for a review on retrograde transport, see Ibanez 2007). Injection of $^{125}$I-GDNF in striatum results in labeled cells in the ipsilateral SN and VTA, thus suggesting a trophic role of GDNF in adult nigrostriatal and mesolimbic neurons (Tomac et al. 1995b).

GDNF signals through extracellular GPI-anchored receptors (GFRα1–4). GDNF have stronger binding activity over GFRα1 but can signal using the other receptors, although with smaller affinity (Trupp et al. 1998). Two GDNF–GFRα1 complexes recruit a dimer of the tyrosine kinase transmembrane protein (RET) (Durbec et al. 1996, Jing et al. 1996, Trupp et al. 1996; Fig. 2). Both GFRα1 and RET receptors present a wider distribution in brain than GDNF, suggesting that GDNF is able to diffuse to contact with not directly adjacent neuronal populations (Trupp et al. 1997). The presence in several brain regions of only one of the two preferred co-receptors is also striking (see below).

GDNF activation of trans-phosphorylation of specific tyrosine residues of RET modulates several intracellular cascades. The pathways activated by RET have been principally studied in embryonic dopamine mesencephalic cells and perinatal sympathetic neurons; however, the mechanisms directly regulated by RET in adult catecholaminergic cells are poorly known. In sympathetic neurons, PI3K/Akt, Src-family kinases, and Erk pathways are activated after GDNF binds to its receptor. These pathways are also activated by other NTFs, leading to neurite outgrowth and neuronal survival (Kaplan & Miller 2000), bringing into question whether different cell types require specific sets of NTFs, or there is a certain degree of redundancy in neurotrophism. Several arguments support GDNF specificity, including its highly restricted expression pattern (Fig. 1); the fact that different cell types require activation by GDNF of distinct intracellular pathways to elicit the same cellular processes and that although signaling by GDNF shows some degree of coincidence with other NTFs, clear dissimilarities are also appreciated (Airaksinen & Saarma 2002). Furthermore, selective removal of GDNF in adult life leads to cell death in specific brain areas (see below). Several modulators of the GDNF/GFRα1/RET pathway have been described. Lipid rafts, a special membrane domain where signaling processes are concentrated, are enriched in GPI-anchored proteins like GFRα1. Upon GDNF stimulation, RET is recruited to lipid rafts, having different signaling partners in each cell compartment (Paratcha & Ibanez 2002). TGF-β is also a modulator of GDNF signaling, contributing to the functional translocation of GFRα1 to cell membrane in several cell types (Kriegstein et al. 1998, Peterziel et al. 2002). GDNF protective action over DA neurons in a MPTP mouse model requires both TGF-β and GDNF, indicating that the cooperative prosurvival effects of both molecules are conserved during adulthood (Schober et al. 2007). The prosurvival effect of TGF-β/GDNF over primary neurons relies on ERK/MAPK pathway and does not require PI3K activity (Peterziel et al. 2002). Heparin sulfate glycosaminoglycans, like syndecans, contribute to modulate the amount of GDNF required for receptor activation. Proteoglycans concentrate GDNF close to GFRα1 and RET receptors, optimizing signal transduction efficiency (reviewed by Sariola & Saarma 2003).

GFRα1 and RET expression patterns are not fully overlapping (Trupp et al. 1997). This observation has lead to the search of alternative GDNF receptors. Several non-canonical forms of GDNF signaling have

![Figure 2](https://www.endocrinology-journals.org)

**Figure 2** Schematic representation of GDNF signaling pathways. Arrows indicate interactions and boxes contain receptor protein domains (top) and cytoplasmic proteins (bottom). Protein domains are not represented scaled.
been described in the adult rodent brain. In the absence of RET, GDNF induces phosphorylation of ERK/MAPK, PLC-γ, and CREB, and Src-family kinases and Fos activation (Poteryaev et al. 1999, Trupp et al. 1999). Met, a tyrosine kinase receptor for the hepatocyte growth factor, has been proposed as a contributor of this RET-independent GDNF signaling. Met activation can be modulated by GDNF using syndecans and the associated Src-type kinase pathway (Fig. 2; Popsueva et al. 2003). Another proposed mechanism for RET-independent signaling relates to the neural cell adhesion molecule (NCAM), a protein belonging to immunoglobulin superfamily (Paratcha et al. 2003). GDNF interacts with low affinity with NCAM but shifts to a high-affinity binding in the presence of GFRz1. NCAM respond to GDNF by activating similar pathways to those elicited by homophilic interactions, including activation of Fyn kinase (Paratcha et al. 2003). GDNF regulates neuronal morphology, cell migration, and synapse formation through the NCAM/GFRz1 complex (for a recent review, see Ibanez 2010). In DA neurons of the SN, integrin αV and NCAM may mediate the phenotypic changes associated with GDNF stimulation in vitro and in vivo (Chao et al. 2003). Recently, it has also been suggested that integrin β1, other cell adhesion molecule, is involved in GDNF signaling (Cao et al. 2008). A ligand-induced cell adhesion mechanism has also been proposed for trans-cellular interaction of GFRz1 molecules in the presence of GDNF. This mechanism has been described in hippocampal neurons and it remains to be determined where it is also of functional relevance for adult DA neurons (Ledda et al. 2007).

GDNF has been shown to activate molecules related with the promotion of antioxidant defense and neuronal survival (for a review, see Saavedra et al. 2008). Among these pathways, the PI3K/AKT cascade (Neff et al. 2002) can protect neurons through several mechanisms including inactivation of apoptotic proteins (Dudek et al. 1997, Soler et al. 1999). GDNF over-expression (using lentiviral infection or engineered GDNF-producing cells) protects catecholaminergic neurons from toxic damage and induces fiber outgrowth in vitro (Arenas et al. 1995, Tomac et al. 1995a, Gash et al. 1996, Choi-Lundberg et al. 1997). However, the molecular mechanisms underlying these functional effects are still largely unknown. Recently, it has been reported that lentiviral GDNF delivered to the rat striatum induces gene expression in the SN, notably tyrosine hydroxylase (TH), GTP cyclohydrolase-I (which catalyzes the synthesis of a cofactor of TH, tetrahydrobiopterin), GDNF receptors, and Dlk-1 (a factor involved in cell proliferation and differentiation). As GDNF receptors are located in terminals of SN neurons, it is presumed that these genes are up-regulated in DA SN neurons in response to GDNF activation (Christophersen et al. 2007).

In recent years, several reports have investigated the role of GDNF in regulating mesolimbic circuits. Dopamine neurons in VTA express high levels of RET and GFRz1, and GDNF is produced in the nucleus accumbens, a VTA target region (Trupp et al. 1997). Infusion of GDNF in VTA blocks the ability of some drugs (cocaine and morphine) to stimulate biochemical changes at the mesolimbic pathway (Messer et al. 2000). These observations, suggesting a role of GDNF as a blocker of the effect of psychostimulants, have been extended to other drugs of abuse like opioids and ethanol (for a review, see Carnicella & Ron 2009). The mechanisms operating to prevent drug-related phenotypes include the activation of MAPK, PI3K, and PLCγ (Carnicella & Ron 2009). In addition to these well-established signaling pathways, GDNF prevents ethanol-mediated association of TH with the chaperone heat-shock protein 90, leading to a restoration of TH protein levels (He & Ron 2008). In any event, GDNF-dependent signaling pathways in brain are, as yet, poorly studied and the GDNF-mediated mechanisms required for the maintenance of adult catecholaminergic neurons remain essentially unknown.

Genetic models of GDNF depletion

Most of the knowledge available on the physiological function of GDNF has come from the analysis of genetically modified mice models. It is more than 15 years since three groups independently reported that ablation of the GDNF gene (Gdnf−/− mice) results in animal death after birth due to renal agenesis and the absence of the enteric plexus (Moore et al. 1996, Pichel et al. 1996, Sanchez et al. 1996). Unexpectedly, the Gdnf−/− mice exhibited an apparently normal number and organization of mesencephalic DA neurons. Embryonic (E14) slice cultures from wt and GDNF−/− animals have shown that, in the absence of GDNF, the neurite outgrowth is inhibited without affecting neuronal survival in vitro (af Bjerken et al. 2007). These observations suggest that the trophic dependence of nigrostriatal neurons on GDNF, supported by the pharmacological experiments (exogenous administration of the trophic factor), might be acquired during postnatal maturation. In support of this view is the fact that blocking GDNF function by injection of neutralizing antibodies enhances cell death during the first period of naturally occurring apoptosis in the SN (Oo et al. 2003). In addition, striatal GDNF expression leads to a transient increase in SN cell number and a permanent augmentation in the number of VTA neurons (Kholodilov et al. 2004).
Heterozygous Gdnf mice are fully viable and develop normally, showing few behavioral alterations during the first postnatal weeks. A group has reported impairment of a hippocampal formation-dependent memory task in 4–8-month old Gdnf<sup>+/−</sup> animals, without alterations in the nigrostriatal pathway (Gerlai et al. 2001). However, memory alterations in animal models of GDNF deficiency have not been analyzed in detail. Partial GDNF deficiency has been associated with a worsening of the phenotypes caused by chronic administration of the drug of abuse, a fact that has lead to the proposal of GDNF as a potential target to treat addiction (for a review, see Carnicella & Ron 2009). Spontaneous motor activity and coordination decline associated with age in heterozygous GDNF animals (Table 1; Boger et al. 2006). This behavioral alteration is paralleled with a small decrease of TH-positive SN neurons at 12 months of age and differences in striatal TH<sup>+</sup> fiber density with respect to controls (Boger et al. 2006). Embryonic GDNF deficit predisposes Gdnf<sup>+/−</sup> mice to higher susceptibility to catecholaminergic neurotoxins when compared with wild-type littermates (Boger et al. 2007). Similar results have been reported in aged GFRα<sub>1</sub>−/− mice (Table 1; Boger et al. 2008, Zaman et al. 2008).

Genetic deletion of the GDNF receptor RET in DA neurons has produced discordant reports regarding the role of this pathway in maintenance of neurons during adulthood. In a first report, no differences in adult (6–12 months of age) DA nigrostriatal neuronal numbers have been observed when RET was conditionally removed from DA neurons, as determined by comparative morphometric and biochemical analysis (Jain et al. 2006). However, a second study using the same genetic approximation has been able to detect a significant decrease of TH<sup>+</sup> SN neurons and striatal fiber density of RET aged mice. Conditional RET deletion did not compromise the viability of neurons in the VTA and LC (Table 1; Kramer et al. 2007). The variable results obtained with the regional RET-null mouse might be related to the fact that, as discussed above, GDNF can signal through ‘non-canonical’ N-CAM receptors (Paratcha et al. 2003), besides the Grfz1/RET pathway, that may compensate for the absence of RET. In both the studies, the Cre line removes RET during embryonic development, a situation that can lead to up-regulation of other GDNF receptors or NTFs. In addition, the study by Jain et al. (2006) could have been hampered by the fact that they pooled animals from 6–8 to 12 months of age in their analysis, which could dilute the subtle differences found by Kramer et al. (2007) in 12-month-old animals.

To address the physiological role of GDNF in catecholaminergic central neurons, we have recently developed a conditional GDNF-null mouse where GDNF expression can be reduced during adulthood, avoiding adaptive compensatory mechanisms that can

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<th>Central catecholaminergic neuron phenotype in animal models of the GDNF signaling pathway.</th>
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<td>Animal model</td>
<td>TH + cells SN</td>
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<td>Reference</td>
<td>TH + cells SN</td>
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<tr>
<td>GDNF&lt;sup&gt;+/−&lt;/sup&gt;</td>
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<td>GFRα&lt;sub&gt;1&lt;/sub&gt;−/−; Dat-Cre&lt;sup&gt;(1)&lt;/sup&gt;</td>
<td>8 m: 85&lt;sup&gt;‡&lt;/sup&gt;</td>
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<td>GDNF&lt;sup&gt;−/−&lt;/sup&gt;; Cre-EsR1&lt;sup&gt;(1)&lt;/sup&gt;</td>
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Numbers indicate percentage with respect to control animals. X m: age at killing (months). X m$: months after GDNF depletion. (dSt) dorsal striatum; (vSt) ventral striatum. n.a., not analyzed; n.s.d., no significant differences. *P < 0.05; †P < 0.01.
occur during development (Pascual et al. 2008). A selective and extensive catecholaminergic neuronal death, most notably in the LC, SN, and VTA, was observed in the conditional GDNF-deficient mice (Table 1 and Fig. 3A). These mice present a catecholaminergic selective phenotype, as other neuronal types analyzed (GABAergic and cholinergic) appear to be unaffected. Other TH-containing neurons, including those in the arcuate nucleus (AN), are not affected by the absence of GDNF, and phenotypes associated with hypothalamic or hypophyseal dysfunction were not observed in the cGDNF animals. The AN is a DA center at the hypothalamus–hypophyseal axis with the peculiarity of having dissociated the synthesis of DA. A significant number of AN neurons produce only one of the enzymes required for DA synthesis (Ugrumov 2009), being the production of DA, a cooperative task involving several neuronal subpopulations. The neurochemical and histological alterations in GDNF-deprived mice leads to the development of behavioral motor abnormalities characterized by a progressive hypokinetic syndrome (Fig. 3B). These data have demonstrated that endogenous GDNF is absolutely required for trophic maintenance of specific DA and noradrenergic neurons. The GDNF-deficient mouse is a well-defined model for studying neuroprotection in experimental PD. The GDNF-controlled molecules that are required for mammalian SN, VTA, and LC neuronal survival remain to be defined.

Clinical effects of GDNF

For several decades, dopamine cell replacement (most frequently intrastriatal transplantation of DA-producing fetal midbrain neurons) has been considered as an experimental therapeutic approach to advanced PD, once pharmaceutical drugs have ceased to provide clinical benefit (Lindvall et al. 1990, Freed et al. 1992, Piccini et al. 1999). The actual clinical efficacy of DA-cell replacement, although as yet a matter not completely settled, has been seriously questioned by controlled, double-blinded clinical trials (Freed et al. 2001, Olanow et al. 2003). Therefore, as an alternative therapeutic strategy to DA-cell replacement, the interest has shifted to the intrastriatal delivery of NTFs that could ‘protect’ nigrostriatal neurons and thus halt or retard PD progression (Deierborg et al. 2008, Vastag 2010). The prototypical ‘neuroprotective’ agent used in most preclinical and clinical studies is GDNF that, as commented in previous sections, has demonstrated a remarkable trophic effect on mesencephalic DA neurons in vitro (Lin et al. 1993) and in vivo (Tomac et al. 1995a,b; for a review, see Kirik et al. 2004).

The good results obtained in the preclinical studies regarding the neuroprotective role of GDNF on catecholaminergic neurons have stimulated the development of clinical trials designed to test the therapeutic effects of GDNF in advanced PD patients. Some groups have assayed the effect of intrastriatal transplantation of GDNF-producing cells, such as those in the carotid body, with moderate results (Arjona et al. 2003). Currently, in vitro carotid body expansion is being tested using animal (Pardal et al. 2007) and human tissue to increase the number of cells available for transplantation (see Minguez-Castellanos et al. 2007). In parallel with these ‘cell therapy’ studies, several clinical trials have been performed using the direct intracerebral infusion of GDNF. In a controlled clinical trial, monthly intraventricular GDNF injection failed to provide clinical benefit in advanced PD patients.
patients and instead resulted in frequent adverse events (Nutt et al. 2003). A post-mortem examination in one patient suggested that GDNF did not reach the target cells via this route. However, encouraging clinical and neurochemical results were observed with continuous intraputaminal GDNF infusion on PD patients in two independent open-label clinical trials. One of the trials performed on five PD patients reported favorable clinical outcomes at 1 year, whereas [18F]-dopa PET studies showed an increase in putaminal uptake around the tip of each catheter (Gill et al. 2003). The second study on ten patients using a different delivery protocol also reported positive results at 6 months (Slevin et al. 2005). However, a randomized placebo-controlled trial involving 34 PD patients showed no significant clinical differences between groups at 6 months, despite increased [18F]-dopa uptake in the recombinant GDNF-treated group (Lang et al. 2006). The open-label extension of this study was halted due to safety concerns: three patients developed neutralizing antibodies, which could potentially cross-react with endogenous GDNF, whereas in a parallel toxicity study, some monkeys developed cerebellar damage. Besides GDNF, other members of the same protein family (particularly neurturin) are also being assayed in pilot clinical trials with as yet inconclusive results.

Concluding remarks

NTFs exert a potent effect on the survival and maintenance of phenotype in adult neurons, therefore intracerebral administration of these factors is a promising therapeutic strategy in neurodegenerative disorders, such as PD, presenting progressive neuronal death. There is a vast scientific literature supporting the neuroprotective role of exogenous GDNF on the nigrostriatal pathway. However, most of the clinical trials performed to test the efficacy of NTF-based therapies in advanced PD patients have been quite discouraging. The generation of the conditional GDNF-null mouse model has recently allowed us to show the absolute requirement of GDNF for survival of DA and noradrenergic neurons in adult brain. These data unequivocally demonstrate a major physiological neuroprotective effect of GDNF and therefore it should reanimate the interest in GDNF-based therapies. Interestingly, DA neurons in the hypothalamus appear to be unaffected by GDNF retrieval.

Clinical application of NTFs is confronted with several technological and scientific challenges that should be addressed in future preclinical and clinical research. Before new clinical trials are performed, a safe and efficacious route of GDNF delivery (either produced in cells, purified, or encoded in viral vectors) must be clearly established. In this regard, diffusion of GDNF in the brain parenchyma and the appropriate concentration of GDNF delivered to cells are critical issues that might determine the clinical outcome. It must also be investigated whether the administration of appropriate cocktails of several trophic factors offer advantages over the use of GDNF alone. Besides these technologically oriented studies, much research should be done to unravel the actual physiological role of GDNF, and other NTFs, and their molecular mechanism of action on adult central neurons. This work might eventually lead to the identification of new signaling pathways that will provide targets accessible to small molecules amenable for their use as pharmaceutical drugs. The molecular physiology and pharmacology of neuroprotection are still at their infancy. Therefore, it can be presumed that the development of these fields will surely offer new opportunities for a more effective fight against PD and other neurodegenerative diseases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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