



Published in final edited form as:

Parkinsonism Relat Disord. 2012 January ; 18(0 1): S143–S146. doi:10.1016/S1353-8020(11)70045-1.

Neurorestoration

Mikko Airavaara^{1,*}, Merja H. Voutilainen^{2,*}, Yun Wang³, and Barry Hoffer³

¹ Institute of Biotechnology, University of Helsinki, Finland ² Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Helsinki, Finland ³ National Institute on Drug Abuse, Intramural Research Program Baltimore, Maryland

Abstract

Although initially thought to be important primarily in neural development, a number of trophic proteins have been found to have neuroprotective and neuroregenerative activity in the adult central system, particularly for midbrain dopamine neurons (MDN). Neurorestoration is potentially feasible for MDN since there is an initial loss of phenotype for these neurons in Parkinson's disease (PD) rather than neuronal death. There is a considerable recent literature on trophic properties of TGF- β superfamily proteins for MDN's, including glial cell-derived neurotrophic factor (GDNF), neurturin, and bone morphogenetic proteins (BMPs). This paper will review studies with the factors listed above, as well as describe more recent studies with two newly described trophic proteins, MANF and CDFN. Data will be presented from various animal models of PD suggesting that these trophic proteins may eventually lead to PD therapeutics in man. In addition, some data on small molecules with neuroprotective properties (AP₄A, retinoic acid and vitamin D₃) will also be described.

Introduction – the concept of neurorestoration in PD

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the cardinal motor symptoms of tremor, rigidity, postural stability and bradykinesia. In PD dopaminergic cells die most prominently in the area of substantia nigra. Current therapies of PD do not prevent the progression of the disease and the efficacy of these treatments wanes over time. In this review we will focus on the potential for neurorestoration therapy in Parkinson's disease. The concept of neurorestoration therapy is based on clinical and PET studies, showing that at the onset of the symptoms there is about 80% decrease in dopamine content in the striatum but about 50% of nigral DA cells are viable [1]. Thus, initially there is loss of the dopamine phenotype. The aim of the neurorestoration therapy is to change the pathophysiological environment towards restoration of the dopamine phenotype. "Neurorestoration" also includes the repopulation of dopamine neurons using cell transplantation or through endogenous neuroprogenitor cells, but these two areas are not the focus of this review. We will focus instead on neurotrophic factors and small molecules. Trophic factors are secreted proteins and are grouped into families based on structural homology, receptors and common signal transduction pathways. We will summarize data on the two most studied neurotrophic factors, glial cell line derived neurotrophic factor (GDNF)

Corresponding author: Barry Hoffer bhoffer@intra.nida.nih.gov.
*equal contribution

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and neurturin, as well as more recently discovered cerebral dopamine neurotrophic factor (CDNF) and mesencephalic astrocyte-derived neurotrophic factor (MANF). The small molecular weight compounds discussed here are diadenosine tetraphosphate (AP₄A) and vitamin D₃.

Neurotrophic Proteins

Glial cell line derived neurotrophic factor (GDNF)

The GDNF family ligands consist of GDNF, neurturin, artemin and persephin and they form a distant group in the TGF- β superfamily [2]. GDNF family members function as homodimers and signal through a transmembrane receptor tyrosine kinase (RET) by first binding to their cognate GDNF family receptor α (GFR α) [2]. In addition to RET, neural cell adhesion molecule (NCAM) has been found to be an alternative receptor for GDNF and syndecan-3 for GDNF and neurturin.

GDNF was purified from a rat glioma cell line (B49) medium and found to promote survival of embryonic dopamine neurons and increase dopamine uptake [3]. GDNF is not specific for dopamine neurons and has been shown to promote survival of several other neuronal populations including motoneurons, noradrenergic neurons, serotonergic neurons, enteric neurons, peripheral sensory and autonomic neurons. The efficacy of recombinant GDNF protein in neurorestoration was first shown in mouse and rat models of PD by us [4] and others [5]. In non-human primate models of PD, GDNF increases the number of dopamine neurons and improves Parkinsonian symptoms such as bradykinesia, rigidity, balance and posture [6]. Overall, these studies suggest that GDNF's most prominent effect is to facilitate regrowth of dopaminergic nerve terminals at the site of administration, and in studies where fluorogold has been used to label dopamine neurons before 6-OHDA injections, it has been suggested that GDNF restores dopaminergic phenotype of injured cells [7].

Gene therapy has also been utilized in studies exploring neuroprotective and neurorestorative effects of GDNF in animal models of PD. Neurorestorative effects of GDNF have been shown by using several different viral vectors and animal models including adeno-associated viral vectors (AAV) in rat models of PD [8], AAV in non-human primates [9], lentivirus in rats [10] and adenovirus [11] in rats.

Although, GDNF was found to be very promising in animal models of PD, the results from clinical trials have been mixed. A study in which GDNF was given ICV reported no improvement and several negative side effects [12]. However, two smaller non-randomized studies where GDNF was delivered directly into putamen showed improvement in motor functions as well as increased dopamine uptake measured by PET without side-effects [13]. A larger randomized trial with thirty four PD patients failed to show improvement but showed an increase in dopamine uptake in the putamen after GDNF infusion. A remaining question is why, despite positive animal model data, the use of GDNF in this clinical trial failed?

Neurturin

Neurturin was originally identified through its ability to promote the survival of cultured sympathetic neurons. Neurturin has been shown to be both neuroprotective and neurorestorative in animal models of PD [14].

Neurturin also entered clinical trials and a viral vector-based platform was chosen to deliver neurturin. In a phase I, open-label clinical trial, the neurturin gene was delivered into the putamen using an AAV vector, but no significant clinical improvement was observed after 12 months. Also a double-blind, randomised trial in 58 patients with advanced PD, using

AAV-neurturin versus sham surgery, did not show significant differences in the primary endpoints [15]. In future studies with AAV-neurturin the SN will be targeted directly, higher doses of AAV-neurturin will be injected to the putamen, and patients will be followed for longer time periods [15].

CDNF and MANF

CDNF (cerebral dopamine neurotrophic factor) and MANF (mesencephalic astrocyte-derived neurotrophic factor) are secreted proteins that constitute a novel, evolutionarily conserved neurotrophic factor family [16]. Both CDFN and MANF have been shown to be both neuroprotective and neurorestorative in animal models of PD. Several mechanisms have been postulated including actions on mitochondrial complex I, endoplasmic reticulum stress, oxidative stress and anti apoptotic effect. Structural analysis suggests that CDFN and MANF may have a dual mechanism of action at the cellular level. The amino-terminal domain is a saposin-like putative lipid-binding domain, suggesting that MANF and CDFN may bind lipids in membranes. The carboxy-terminal domain of CDFN and MANF may protect cells against ER stress.

In the first study a single intrastriatal injection of CDFN (10 μ g) restored the function of dopaminergic neurons in the SNpc and prevented their degeneration [16]. In the neurorestoration experiment CDFN was injected into the same location in striatum as was 6-OHDA four weeks later. CDFN significantly reduced abnormal rotational behavior and the number of TH-positive cells in the SNpc was higher in these rats. In a follow-up study a 14-day continuous infusion of CDFN was able to protect nigrostriatal dopaminergic nerves from 6-OHDA-induced degeneration and restore the functional balance of the nigrostriatal neural circuits as assessed by morphological and behavioral analyses [17]. A 14-day continuous infusion of CDFN was also able to restore 6-OHDA-induced loss of the TH-positive DA phenotype. A third study of CDFN where both neuroprotective and neurorestorative paradigms were used involved the mouse MPTP model. In this study we showed that intrastriatally administered CDFN protects the dopamine neurons phenotype when administered 1 day before MPTP and restored the dopamine neurons phenotype when give 1 week after MPTP [18]. This effect was shown both histochemically in dopamine neurons and neurites as well with behavioral measurements.

Intrastriatal MANF (10 μ g) was able to restore the functional activity of the 6-OHDA lesioned nigrostriatal dopaminergic system and the maximum effect was evident at 12 weeks post-lesion. Consistent with results from the behavioral studies, MANF was also able to partially restore TH-positive cell bodies in the SNpc as compared to the vehicle-treated controls [17]. It should be emphasized that most trophic factors in the TGF- β superfamily have a sharp “inverted U” dose response curve with lesser efficacy at both lower and higher levels. This is a critically important consideration for clinical development. Table 1 summarizes similarities and differences between neurotrophic factors GDNF, NRTN, CDFN and MANF from a potential clinical perspective.

Small molecules for Parkinson's disease

Two examples of the restorative actions of small molecules will be described here: AP₄A and Vitamin D₃.

Diadenosine tetraphosphate—Diadenosine tetraphosphate (AP₄A) is a compound that contains two adenosine moieties bridged by 4 phosphates. Selective AP₄A binding sites are found in substantia nigra and striatum.

AP₄A has protective effects on dopaminergic neurons. In primary ventromesencephalic (VM) neuronal cultures, the density of tyrosine hydroxylase (TH) neurons and fibers were significantly reduced while caspase-3 activity was enhanced at 2 days after application high doses of methamphetamine (MA), a dopaminergic neurotoxin. Pretreatment with AP₄A attenuated the MA-mediated decrease in TH fiber density in VM cultures, suggesting that AP₄A is neuroprotective in VM cells. Similarly, AP₄A also reduced the methamphetamine-mediated decrease in TH immunoreactivity in SNpr *in vivo*.

A more direct protective response to AP₄A was reported using a rat model of PD by unilaterally lesioning DA neurons with 6-hydroxydopamine (6-OHDA). One month after lesioning, vehicle-treated rats exhibited amphetamine-induced rotation. Minimal tyrosine hydroxylase immunoreactivity was detected in the lesioned nigra and striatum and no KCl-induced dopamine release was found. All of these indices of a dopaminergic lesion were attenuated by pretreatment with AP₄A. In addition, AP₄A reduced TUNEL labeling in the lesioned nigra two days after 6-OHDA administration. These data suggest that AP₄A is protective against neuronal injuries induced by 6-OHDA through the inhibition of apoptosis [19].

1,25 dihydroxyvitamin D₃—1,25-dihydroxyvitamin D₃ (D₃), an active metabolite of vitamin D, is a potent inducer of GDNF. D₃ augments GDNF expression in C6 glioma cells and GDNF release in human U-87MG glioblastoma cells. D₃ also increases nerve growth factor (NGF), increases transforming growth factor (TGF)-2 expression in neuroblastoma cells, and elevates NT3/NT4 mRNA levels in astrocytes. Pretreatment with D₃ increases GDNF levels in rat brain [20]. These data suggest that D₃ upregulates GDNF and other trophic factors *in vivo* and *in vitro*. Moreover, since D₃, unlike GDNF, is able to cross the blood brain barrier, it is possible that systemic administration of this compound could restore DA circuits indirectly via an elevation of endogenous trophic factors in brain.

In *in vitro* VM cultured neurons, D₃ (10⁻¹⁰ M) reduced 6-OHDA or H₂O₂-induced cell death. Pretreatment with D₃ for 8 days significantly restored locomotor activity in unilateral 6-OHDA-lesioned rats. D₃ also protected against 6-OHDA-mediated depletion of DA and its metabolites in substantia nigra [20]. Taken together, these data indicate that D₃ pretreatment attenuates the hypokinesia and DA neuronal toxicity induced by 6-OHDA. Since both H₂O₂ and 6-OHDA may injure cells via free radical and reactive oxygen species, the neuroprotection seen here may operate via a reversal of such a toxic mechanism.

Conclusions and future directions

Several questions remain for future studies [21, 22]. Given the size differences between rodent, non-human primate and the human brains, studies on delivery techniques must be undertaken. Since neurotrophic factors cannot pass through the blood-brain barrier, they must be administered directly intracranially, which can cause similar adverse effects as has been reported with deep brain stimulation; such as surgery related complications, hardware related problems as well as long-term effects on behavioral disorders [23]. Would it be best to deliver as a single bolus, by continuous infusion or via convection-enhanced delivery? What type of cannula design would allow optimum dose and spread of these factors? What would be the best site for delivery, caudate/putamen or nigra? In addition, would viral vector-based delivery methods produce a long lasting and safe way to delivery drug? Is there a need for cell specificity and regulated on/off-systems when viral vectors are used? At what stage of human PD should neurotrophic factors be delivered? If one waits for a late stage, would there be enough nigrostriatal projections remaining to allow intrastriatal delivery to be retrogradely transported back to nigra. Finally, is there a role for small molecule therapeutics that work by elevating endogenous neurotrophic factors or by activating other

transduction mechanisms? These complexities notwithstanding, neurorestorative therapeutic strategies may provide a unique approach to reverse progression of this devastating illness.

References

1. Leenders KL, Salmon EP, Tyrrell P, Perani D, Brooks DJ, Sager H, et al. The nigrostriatal dopaminergic system assessed *in vivo* by positron emission tomography in healthy volunteer subjects and patients with Parkinson's disease. *Arch Neurol*. 1990; 47:1290–8. [PubMed: 2123623]
2. Airaksinen MS, Saarma M. The GDNF family: signalling, biological functions and therapeutic value. *Nat Rev Neurosci*. 2002; 3:383–94. [PubMed: 11988777]
3. Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science*. 1993; 260:1130–2. [PubMed: 8493557]
4. Hoffer BJ, Hoffman A, Bowenkamp K, Huettl P, Hudson J, Martin D, et al. Glial cell line-derived neurotrophic factor reverses toxin-induced injury to midbrain dopaminergic neurons *in vivo*. *Neurosci Lett*. 1994; 182:107–11. [PubMed: 7891873]
5. Rosenblad C, Kirik D, Devaux B, Moffat B, Phillips HS, Bjorklund A. Protection and regeneration of nigral dopaminergic neurons by neurturin or GDNF in a partial lesion model of Parkinson's disease after administration into the striatum or the lateral ventricle. *Eur J Neurosci*. 1999; 11:1554–66. [PubMed: 10215908]
6. Gash DM, Zhang Z, Ovadia A, Cass WA, Yi A, Simmerman L, et al. Functional recovery in parkinsonian monkeys treated with GDNF. *Nature*. 1996; 380:252–5. [PubMed: 8637574]
7. Bowenkamp KE, David D, Lapchak PL, Henry MA, Granholm AC, Hoffer BJ, et al. 6-hydroxydopamine induces the loss of the dopaminergic phenotype in substantia nigra neurons of the rat. A possible mechanism for restoration of the nigrostriatal circuit mediated by glial cell line-derived neurotrophic factor. *Exp Brain Res*. 1996; 111:1–7. [PubMed: 8891630]
8. Kirik D, Rosenblad C, Bjorklund A, Mandel RJ. Long-term rAAV-mediated gene transfer of GDNF in the rat Parkinson's model: intrastriatal but not intranigral transduction promotes functional regeneration in the lesioned nigrostriatal system. *J Neurosci*. 2000; 20:4686–700. [PubMed: 10844038]
9. Eberling JL, Kells AP, Pivrotto P, Beyer J, Bringas J, Federoff HJ, et al. Functional effects of AAV2-GDNF on the dopaminergic nigrostriatal pathway in parkinsonian rhesus monkeys. *Hum Gene Ther*. 2009; 20:511–8. [PubMed: 19254173]
10. Brizard M, Carcenac C, Bemelmans AP, Feuerstein C, Mallet J, Savasta M. Functional reinnervation from remaining DA terminals induced by GDNF lentivirus in a rat model of early Parkinson's disease. *Neurobiol Dis*. 2006; 21:90–101. [PubMed: 16084732]
11. Smith AD, Kozlowski DA, Bohn MC, Zigmond MJ. Effect of AdGDNF on dopaminergic neurotransmission in the striatum of 6-OHDA-treated rats. *Exp Neurol*. 2005; 193:420–6. [PubMed: 15869944]
12. Kordower JH, Palfi S, Chen EY, Ma SY, Sendra T, Cochran EJ, et al. Clinicopathological findings following intraventricular glial-derived neurotrophic factor treatment in a patient with Parkinson's disease. *Ann Neurol*. 1999; 46:419–24. [PubMed: 10482276]
13. Gill SS, Patel NK, Hotton GR, O'Sullivan K, McCarter R, Bunnage M, et al. Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med*. 2003; 9:589–95. [PubMed: 12669033]
14. Oiwa Y, Yoshimura R, Nakai K, Itakura T. Dopaminergic neuroprotection and regeneration by neurturin assessed by using behavioral, biochemical and histochemical measurements in a model of progressive Parkinson's disease. *Brain Res*. 2002; 947:271–83. [PubMed: 12176170]
15. Marks WJ Jr, Bartus RT, Siffert J, Davis CS, Lozano A, Boulis N, et al. Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol*. 2010; 9:1164–72. [PubMed: 20970382]
16. Lindholm P, Voutilainen MH, Laurén J, Peränen J, Leppänen VM, Andressoo JO, et al. Novel neurotrophic factor CDNF protects and rescues midbrain dopamine neurons *in vivo*. *Nature*. 2007; 448:73–7. [PubMed: 17611540]

17. Voutilainen MH, Back S, Peranen J, Lindholm P, Raasmaja A, Mannisto PT, et al. Chronic infusion of CDFN prevents 6-OHDA-induced deficits in a rat model of Parkinson's disease. *Exp Neurol*. 2011; 228:99–108. [PubMed: 21185834]
18. Airavaara M, Harvey BK, Voutilainen MH, Shen H, Chou J, Lindholm P, et al. CDFN protects the nigrostriatal dopamine system and promotes recovery after MPTP treatment in mice. *Cell Transplant*. 2011 in press.
19. Wang Y, Chang CF, Morales M, Chiang YH, Harvey BK, Su TP, et al. Diadenosine tetraphosphate protects against injuries induced by ischemia and 6-hydroxydopamine in rat brain. *J Neurosci*. 2003; 23:7958–65. [PubMed: 12944527]
20. Wang JY, Wu JN, Cherng TL, Hoffer BJ, Chen HH, Borlongan CV, et al. Vitamin D(3) attenuates 6-hydroxydopamine-induced neurotoxicity in rats. *Brain Res*. 2001; 904:67–75. [PubMed: 11516412]
21. Chiocco MJ, Harvey BK, Wang Y, Hoffer BJ. Neurotrophic factors for the treatment of Parkinson's disease. *Parkinsonism Relat. Disord*. 2007; 13 S3:S321–8. [PubMed: 18267258]
22. Ramaswamy S, Kordower JH. Are growth factors the answer? *Parkinsonism Relat. Disord*. 2009; 15 S3:S176–80. [PubMed: 20082985]
23. Wolters, ECh. Deep brain stimulation and continuous dopaminergic stimulation in advanced Parkinson's disease. *Parkinsonism Relat. Disord*. 2007; 13 S1:S18–23. [PubMed: 17702631]

Table 1

	GDNF	NRTN	CDNF	MANF
Molecular weight	Homodimer 32 kDa	Homodimer 25 kDa	Monomer 18kDa	Monomer 18kDa
pI	9.5	9.0	7.7	8.6
Binding to heparin and ECM	strong	very strong	low	low
Diffusion in the rat brain	limited	very limited	relatively good	good
Retrograde transport Form STR to SNpc	yes	yes	yes	no
Neuroprotection in 6-OHDA model	+++	+++	+++	+++
Neuroprotection in MPTP model	+++	+++	+++	Not tested
Neurorestoration in 6-OHDA model	+++	+++	+++	+++
Neurorestoration in MPTP model	++	++	+++	Not tested
Neurorestoration in severe 6-OHDA model with continuous infusion of NTFs	Trend, not significant	N/A	++	No effect