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Review

Therapeutic potential of the endoplasmic reticulum located and secreted CDNF/MANF family of neurotrophic factors in Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder where dopamine (DA) neurons in the substantia nigra degenerate and die. Since no cure for PD exists, there is a need for disease-modifying drugs. Glial cell line-derived neurotrophic factor (GDNF) and related neurturin (NRTN) can protect and repair DA neurons in neurotoxin animal models of PD. However, GDNF was unable to rescue DA neurons in an α-synuclein model of PD, and both factors have shown modest effects in phase two clinical trials. Neurotrophic factors (NTFs), cerebral DA NTF (CDNF) and mesencephalic astrocyte-derived NTF (MANF) form a novel family of evolutionarily conserved, endoplasmic reticulum (ER) located and secreted NTFs. CDNF and MANF have a unique structure and an unparalleled dual mode of action that differs from other known NTFs. Both protect cells from ER stress, and regulate the unfolded protein response via interacting with chaperons, and CDNF dissolves intracellular α-synuclein aggregates. By binding to putative plasma membrane receptors, they promote the survival of DA neurons similarly to conventional NTFs. In animal models of PD, CDNF protects and repairs DA neurons, regulates ER stress, and improves motor function more efficiently than other NTFs.

1. Introduction

Parkinson's disease (PD) is an age-related, progressive neurodegenerative disorder characterized by the cardinal motor symptoms of tremor, muscle rigidity, bradykinesia, and postural instability. These motor symptoms of PD result from the degeneration and death of neurons in the substantia nigra (SN) in midbrain, which leads to a loss of dopamine (DA) in the striatum and disrupts the neural circuitry that controls movement [1].

The incidence of neurodegenerative diseases, such as PD, increases with age, causing increasing costs for society. There is no treatment for PD which could prevent the cell loss or halt the progression of the disease. The ideal candidate therapy would be the one which prevents neurodegeneration and restores DAergic circuitry in the brain, thereby halting the progression of debilitating disease symptoms.

During development neurotrophic factors (NTFs) regulate neuronal survival, differentiation and maturation, as well as neurite growth and branching [2]. In adult animals they control metabolic maintenance of the neurons but also protect and repair injured neurons [2]. Because of the therapeutic potential of NTFs for neurological disorders, research in this area has been growing rapidly and a large number of NTFs and their receptors have been identified [3–5].

NTFs have been explored as a novel treatment for PD, where all current treatments are symptomatic and no disease-modifying therapy exists [2]. It should be noted that many NTFs can halt the PD symptoms in in vivo models of PD, but only a few restore DAergic circuitry. The concept to restore the nigrostriatal circuitry is largely based on the findings that degeneration occurs in a dying-back manner, starting from nerve endings in the caudate putamen and is followed by axon and cell body degeneration in the SN [6]. Indeed, at the onset of first motor symptoms the deficits are larger in the putamen than in the midbrain [7,8]. Moreover, it was indicated that at the onset of first motor PD symptoms only 30% of SN pars compacta (SNpc) DA neurons are lost [9]. Thus, at the onset of symptoms more than 50% of DA neurons are viable and neurorestorative properties (i.e. can protect and regenerate DA neurons when applied after the lesion) are an essential opportunity for potential PD therapy.

This review is focused on the two families of NTFs that have shown to be the most promising in their neurorestorative effects in animal models of PD. These factors are glial cell line-derived neurotrophic factor (GDNF) family ligands GDNF and neurturin (NRTN) as well as cerebral dopamine neurotrophic factor (CDNF)

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and mesencephalic astrocyte-derived neurotrophic factor (MANF) from CDNF/MANF family. Brain-derived neurotrophic factor (BDNF) was the first protein identified that directly support the survival of DA neurons in vivo. However, only GDNF, NRTN, MANF and CDNF have well-established neurorestorative properties in the nigrostriatal DAergic system in animal models of PD. Since BDNF and most of the tested growth factors do not protect and repair nigrostriatal neurons after the lesion in animal models of PD, they do not have therapeutic potential in the disease.

Vascular endothelial growth factor A (VEGF-A) and VEGF-B are also promising NTFs and are neuroprotective and restorative in 6-hydroxydopamine (6-OHDA) model of PD. However, these factors have not been tested in non-human primate models of PD or in clinical trials. We have studied the effects of VEGF-C in rat 6-OHDA neuroprotective model, but its effect was rather modest [10].

GDNF has been the most promising NTF studied so far, as GDNF has been able to protect and repair DA neurons in the 6-OHDA and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD in rodents and non-human primates (NHP) [11–13]. However, GDNF showed no clinical benefit in two phase II clinical trials, and related factor NRTN gene therapy had a very modest clinical benefit. Moreover, GDNF did not protect DA neurons in two α-synuclein models of PD [14,15], which may be problematic since α-synucleinopathies are one of the hallmarks in the pathophysiological brain samples of PD patients.

We have recently discovered CDNF, [16] that together with the related factor MANF [17,18], forms a novel conserved NTF family (Fig. 1A, [19,20]). MANF promotes survival of DA neurons in vitro [18], and the invertebrate orthologue DmMANF has been shown to be crucial for maturation and maintenance of the fruit fly (*Drosophila melanogaster*) nervous system [21]. We have shown the therapeutic potential of CDNF in the rat 6-OHDA and mouse MPTP models of PD and found that CDNF is a potent NTF to protect DA neurons and more importantly restore their function [16,22,23]. Furthermore, in the severe rat 6-OHDA model of PD CDNF was more effective than GDNF [23]. CDNF and the related MANF are stable proteins, which diffuse better than any other tested trophic factor in brain tissue [23,24]. Our present understanding of the molecular mechanism of action of CDNF and MANF suggest that they are involved in the regulation of endoplasmic reticulum (ER) stress and unfolded protein response (UPR) [19,25]. In a recent study we discovered that lack of MANF in vivo in mouse leads to chronic activation of UPR [25]. In addition, extracellular CDNF rescues only the neurons that degenerate via ER stress (Krieglstein, Saarma, submitted). Thus, CDNF and MANF have significant potential as a treatment of PD.

Our hypothesis is that CDNF can rescue DA neurons by blocking neuronal apoptosis at least partially by regulating the ER stress response. The exact survival-promoting mechanism of CDNF and MANF is not yet fully solved, but recent data from us [19,25] and others [26] indicate that CDNF and MANF are critically involved in the regulation of ER stress. Thus, their mechanism of action is drastically different from other known NTFs. They can act like classical NTFs by extracellularly promoting cell survival via activating PI3K-Akt pathway (Krieglstein, Saarma, submitted) and possibly other pathways. However, differently from other NTFs, CDNF and MANF are mainly located in the ER, regulate ER stress and UPR intracellularly, but can be secreted from the cells to extracellular space mostly upon ER stress. ER stress is an important pathway that regulates cell homeostasis, but may also lead to cell death in PD. Recent studies of autopsied tissue samples from PD patients, and in vivo experiments with tissue from a PD model in rodents indicate that ER stress is involved in the pathogenesis of PD [27]. Therefore, in addition to an extracellular survival promotion mechanism that is similar to conventional NTFs, CDNF/MANF efficacy in neurodegenerative diseases is likely also related to their ER stress-relieving properties, that is different from other known NTFs.

2. A novel CDNF/MANF family of proteins with unique structure

2.1. Three known classes of neurotrophic factors

NTFs are small secretory proteins that, by binding to their specific receptors, regulate the development, survival and maintenance of neurons. During early stages of development, NTFs are involved in the differentiation and migration of neuronal precursors and during target innervation they act as target-derived NTFs regulating the survival of neurons and through that determine the final number of neurons and the density of innervation. In the adult nervous system NTFs maintain healthy neurons, protect them from toxins and injury, and regulate neuronal plasticity. Three major classes of NTFs have been described. The first discovered and the best studied is neurotrophin family that consist of nerve growth factor (NGF), BDNF, neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4), which regulate neuronal survival and plasticity by activating transmembrane receptor tyrosine kinase (RTK) of the Trk family. These factors and their pro-forms also bind to the p75 neurotrophic receptor and trigger neuronal death. Neurotrophins have a vast variety of effects on various neuronal populations in the peripheral nervous system (PNS) and central nervous system (CNS). BDNF (Fig. 2) can support the survival of embryonic DA neurons and protect them from death in neurotoxin models of PD. However, BDNF is not protecting and repairing DA neurons when added to the midbrain after the neurotoxin lesion. Moreover, its receptor knockout (KO) animals have intact midbrain DA system and BDNF has not been tested in clinical trials on PD.

The family of neurokines, also known as neuropoietic cytokines, are small, structurally related secretory proteins that all signal via transmembrane gp130 receptor. This family includes ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), leukemia inhibitory factor (LIF), neuropoietin (NPN), oncostatin M (OSM), cardiotrophin-like cytokine (CLC), interleukin 6 (IL-6), IL-11 and IL-27. Neurokines use either a two or three component receptor system and support mostly the survival of motoneurons (MNs) [28]. However, the biological effects of neurokines in the nervous system are much less studied than other NTFs. In addition to MNs, CNTF also has survival promoting effects on DA neurons and parasympathetic neurons, and LIF supports sensory neurons.

For the DA neurons the most important factors belong to the GDNF family ligands (GFLs). GDNF and related growth factors NRTN, artemin (ARTN) and persephin (PSPN) form a distant family in the transforming growth factor-β (TGF-β) superfamily of growth factors [29–31]. GFLs specifically bind to GPI-anchored co-receptors of the GFRα family and the GFL–GFRα complex binds to and activates RET receptor tyrosine kinase. GDNF that binds to GFRα1 and NRTN that binds to GFRα2 support the survival of DA, but also have wide effects on different neuronal populations in the CNS and PNS [29] (Fig. 2). Most remarkably, GDNF is absolutely required for the development of the enteric nervous system and together with NRTN they are critical regulators of the development of parasympathetic neurons [32]. NRTN and its receptor GFRα2 KOs have defects in the parasympathetic nervous system, but their brain DA system is completely intact. GDNF, GFRα1 and RET knockouts die at birth because of the lack of kidney, but without defects in the DA system [29]. However, conditional deletion of GDNF in two laboratories has produced completely different results. Pascual et al. [33] removed GDNF from adult mice using tamoxifen induction and reported significant loss of DA neurons in the midbrain resulting is motor disorders. The study concluded that GDNF is critically important for the maintenance and function of DA neurons in adult animals. Our laboratory developed a mouse model