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REVIEW

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Recent progress in studies of neurotrophic factors and their clinical implications

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Abstract Neurotrophic factors are endogenous soluble proteins that regulate long-term survival and differentiation of neurons of the peripheral and central nervous systems. These factors play an important role in the structural integrity of the nervous system, and therefore are good candidates as therapeutic agents for neurodegenerative diseases. However, recent studies have revealed some unexpected, novel roles of neurotrophic factors. Of particular significance is the discovery of the new functions of brain-derived neurotrophic factor (BDNF) and glia-derived neurotrophic factor (GDNF). Physiological experiments indicate that BDNF may serve as regulatory factors for synaptic transmission as well as for learning and memory. Gene targeting studies demonstrate that GDNF may be essential for development of the enteric nervous system (ENS) and kidney organogenesis. These results not only provide new insights into our understanding of the function of neurotrophic factors but may also have significant implications in the therapeutic usages of neurotrophic factors.

Key words Brain-derived neurotrophic factor · trk receptor · Long-term potentiation · Glia-derived neurotrophic factor · c-ret tyrosine kinase · Dopaminergic neurons · Kidney organogenesis · Enteric nervous system · Gene knockout · Hirschsprung's disease

Abbreviations *ALS* Amyotrophic lateral sclerosis · *BDNF* Brain-derived neurotrophic factor · *CNTF* Ciliary neurotrophic factor · *ENS* Enteric nervous system · *GDNF* Glia-derived neurotrophic factor · *LIF* Leukemia inhibitory factor · *LTP* Long-term potentiation · *NGF* Nerve growth factor · *NT* Neurotrophin

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Introduction

According to the classical definition, neurotrophic factors are endogenous soluble proteins that regulate the long-term survival and differentiation of neurons [1]. It is now generally accepted that they are signaling molecules important for both the development and the maintenance of structural integrity within the peripheral and central nervous systems. Extensive studies have been carried out, and significant progress has been made in the past decade. Many potent neurotrophic factors have been discovered. Their receptors have been identified, and great ef-

forts have been made to elucidate their signal transduction mechanisms. The expression and cellular distribution of these factors and their respective receptors in the nervous system have been extensively investigated, and these studies have provide clues for the cell populations that synthesize or respond to particular kinds of neurotrophic factors. Several approaches have been employed to determine the biological function of neurotrophic factors. In many cases new neurotrophic factors were identified using cultures of a specific population of neurons.

Since the microenvironment of neurons in culture is rigorously controlled, it is easy to test the factor's ability to enhance neuronal survival and differentiation and to protect neuronal death due to toxic agents or insults. The function of the neurotrophic factor can also be examined in animals in vivo. The factor is delivered to specific regions of the nervous system to determine its ability to rescue naturally occurring cell death (apoptosis) or to protect against experimentally induced lesions or damage. In recent years the new technology of gene targeting in mice has been used to study the function of neurotrophic factors [2]. In these studies the gene for a particular neurotrophic factor or its receptor has been mutated through homologous recombination in embryonic stem cells, and the consequence of the gene ablation is manifested in the homozygote offspring carrying the mutated alleles in the animal's genome. The mutant mice are studied using physiological, biochemical, morphological, and behavioral approaches. Thus these knockout mice are excellent animal models to study the function of neurotrophic factors.

Several classes of neurotrophic factors have been identified. The prototype is called neurotrophin, which is a family of structurally related secretory proteins that are widely expressed in neurons and their target cells [3, 4]. To date five members have been identified. They are called nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin (NT) 3, 4/5, and 6. These are signaling molecules that function by binding to their respective receptors on the cell surface. NGF binds to TrkA; BDNF and NT-4/5 to TrkB, and NT-3 to TrkC. A second class of neurotrophic factors is the ciliary neurotrophic factor (CNTF) family, which includes CNTF, leukemia inhibitory factor (LIF), interleukin (IL) 6 and 11, and cardiotrophin 1 [5, 6]. Their receptors consist of several components, a common subunit for all members of CNTF family, and other subunits unique for their respective factors [7].

Recently a novel molecule called glia cell line-derived growth factor (GDNF) was discovered for its potent effect on the survival of mesencephalic dopaminergic neurons [8]. GDNF is a distant member of transforming growth factor β superfamily. However, unlike other members in the family, GDNF acts on receptor tyrosine kinase rather than serine/theronine kinases. The receptors for GDNF, similar to the CNTF family molecules, are comprised of multiple components. These include a signaling component called c-ret, an orphan receptor tyrosine kinase [9, 10], and a high-affinity ligand binding component, GDNFR-α [11, 12]. Additional members of the GDNF gene family and GDNFR-α are being cloned and characterized.

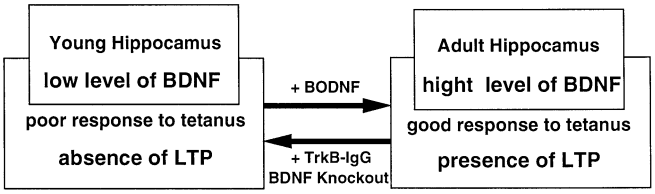

Fig. 1 Role of BDNF in tetanus-induced LTP during hippocampal development

Since chronic neurodegenerative diseases and acute nervous system injury result in structural damage, there has also been a great deal of interest in the use of neurotrophic factors as therapeutic agents for diseases of the nervous system [13]. Substantial evidence indicates that neurotrophic factors can protect and even restore impaired functions resulting from trauma, aging, toxic agents, and genetically linked neurodegenerative disorders. Clinical trials are now in progress to assess the therapeutic efficacy of neurotrophic factors in a variety of neurodegenerative diseases. These clinical studies are based primarily on functional studies in animals. For example, in both rodents and monkeys GDNF has been shown to enhance the survival and protect against the death of dopaminergic neurons in substantia nigra neurons which project to the striatum [14–16]. These neurons are involved in motor function and are degenerated in patients with Parkinson's disease. GDNF therefore is an attractive candidate drug for Parkinson's disease, and clinical trials are underway. Thus the discovery of new functions for a neurotrophic factor may lead to new clinical use of the factor. On the other hand, new findings in animal studies may also provide insights into the potential side effects.

Despite rapid advances in research on neurotrophic factors functional studies have concentrated largely on their roles in neuronal survival and differentiation [1, 17]. However, the recurring observation that the expression of many neurotrophic factors in the central nervous system is rapidly enhanced by neuronal activity [18] suggests a new role for these factors in activity-dependent processes, such as synaptic development and plasticity [19]. Indeed, a number of recent experiments support this hypothesis. Moreover, expression of the receptors for the neurotrophic factors in several nonneuronal tissues raises the possibility that these factors may have a role outside the nervous system. These studies have challenged the classical view of neurotrophic factors, which defines neurotrophic factors as secretory proteins that regulate survival and differentiation of neurons in the central and peripheral nervous systems. This review focuses on the novel and unexpected functions of the neurotrophic factors BDNF and GDNF, These functions cannot be explained by the traditional concept of neurotrophic factors.

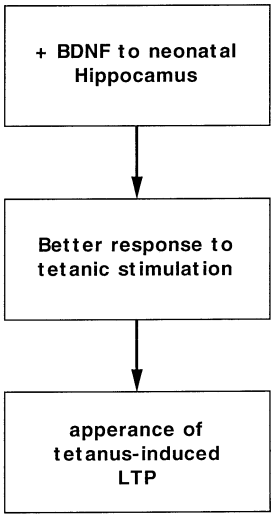

Fig. 2 Mechanism by which BDNF regulates tetanus-induced LTP in developing hippocampus

Role of BDNF in learning and memory

Substantial evidence indicates that the expression of neurotrophin genes in neurons can be regulated by neuronal activity. Activity-dependent regulation of NGF and BDNF mRNAs have been observed in hippocampal neurons in culture [20–23] and in the visual cortex in vivo [24]. Moreover, the levels of NGF and BDNF mRNA increase rapidly in response to kindling as well as recurrent limbic seizures and kainate-induced seizures [25–28]. A recent study demonstrated that in BDNF mutant mice the development but not maintenance of kindling is suppressed [29] (but see [30]). This result implies that BDNF facilitates kindling epileptogenesis in the hippocampus by enhancing synaptic transmission. For a long time it has been speculated that the rapid increase in neurotrophin gene expression may play a role in modulating synaptic transmission or synaptic plasticity [17, 31]. The first direct demonstration of neurotrophic regulation of synaptic activity was the acute effect of neurotrophins on synaptic transmission at the neuromuscular junction [32].

It has been reported that within a few minutes of exposure to the neurotrophins BDNF or NT-3 there is a dramatic increase in the frequency of spontaneous synaptic currents and in the amplitude of evoked synaptic currents. Detailed analyses indicate that this effect is a result of the enhancement of transmitter release, most likely due to increased intracellular calcium concentrations [33, 34]. The acute effects of neurotrophins on neuronal activity and synaptic transmission have also been observed in developing CNS neurons in culture by a number of laboratories [35–38]. These experiments have established that (a) the expression of neurotrophin genes can be regulated by neuronal activity, and (b) neurotrophic factors are capable of acutely enhancing synaptic transmission. The physiological significance of these findings has not been well established. A positive feedback hypothesis has been put forward: neuronal impulse activity enhances the production and secretion of neurotrophins, which in turn serve as retrograde messengers to potentiate neuronal activity and synaptic efficacy [35].

This reciprocal interaction hypothesis strongly suggests a role for neurotrophins in activity-dependent processes such as learning and memory [35]. The best cellular model for learning and memory is the long-term potentiation (LTP) of synaptic efficacy in the hippocampus [39, 40]. The hippocampus is a brain structure known to be involved in the initial retention of information, because damage to this region interferes with the formation of new memory while old memories are not affected. Hippocampal LTP is induced by a brief tetanic stimulation, and the increase in synaptic efficacy can last for hours or even days. The LTP process can be divided into an initial “induction” stage and a later “maintenance” stage [39]. In both the CA1 region and the dentate gyrus of hippocampus the induction of LTP involves a sustained, tetanus-induced depolarization which causes a large influx of Ca^{2+} through NMDA-type glutamate receptors and Ca^{2+} channels. Subsequent biochemical processes triggered by this Ca^{2+} influx have been a subject of intensive studies.

Substantial evidence indicates that the long-term maintenance of LTP [41, 42], just as long-term memory [43], requires gene transcription and protein synthesis. Which genes are those turned on during LTP? The LTP-inducing tetanic stimulation has been shown to enhance the expression of neurotrophin genes, suggesting that neurotrophins play a role in LTP maintenance. In hippocampal slices the application of tetanus to Schaffer collaterals significantly increases BDNF and NT-3 mRNAs in CA1 neurons [44]. Tetanic stimulation of the perforant path in vivo also elicits an increase of mRNAs for NGF and BDNF in granular neurons of dentate gyrus [45, 46]. It has been speculated that neurotrophins are involved in the maintenance of LTP, either by modulating postsynaptic glutamate receptors or by serving as retrograde messengers that regulate presynaptic transmitter release.

If neurotrophins are truly important for LTP, deletion of neurotrophin genes should impair the LTP process. Indeed, in BDNF knockout mice there is a severe defect in the hippocampal LTP, although brain morphology, basal synaptic transmission, and the behavior of these animals appear to be normal [47, 48]. It appears that hippocampal BDNF must maintain a critical level for the expression of LTP. Adult heterozygotes in which the BDNF gene activity has been reduced to half exhibit the same degree of impairment in LTP as those from homozygous animals. However, in animals younger than P16, homozygotes show more severe impairment than heterozygotes, indicating that younger animals are more susceptible to changes in BDNF levels. The defect in hippocampal LTP can be rescued by introducing exogenous BDNF back into the hippocampus of BDNF knockout mice. LTP is completely restored in hippocampal slices from BDNF knockout mice after prolonged incubation with recombinant BDNF [48] or by infection with BDNF-containing adenovirus [49]. These experiments indicate

that the absence of BDNF per se, rather than cumulative developmental defects, is responsible for impaired LTP in BDNF knockout mice.

In the developing hippocampus the expression of BDNF and its receptor TrkB [50–53] increase in parallel with the ability to undergo LTP [54–56]. Interestingly, the ability to learn and remember also improves progressively throughout childhood. In a recent study BDNF was found to accelerate the appearance of tetanus-induced LTP in the developing hippocampus, suggesting that this neurotrophin promotes memory in young animals [57]. In neonatal hippocampal (p12-13) slices tetanic stimulation induced a typical short-term potentiation (STP) lasting less than 30–40 min. In contrast, the same tetanic stimulation elicited a stable LTP in slices treated with BDNF. The effect of BDNF in the neonatal hippocampus was specific. Neither NGF nor NT-3, which activate TrkA and TrkC receptor tyrosine kinases, respectively, had any effects on LTP development. Moreover, NT-4/5, which acts on the same TrkB receptor, had an effect similar to that of BDNF. The BDNF effect was prevented by pretreatment of the slices for 20–30 min with a TrkB-IgG fusion protein, a specific scavenger for BDNF and NT-4/5 [58]. Thus the BDNF effect was mediated by TrkB receptors. In the adult hippocampus, which normally expresses high levels of endogenous BDNF and TrkB, exogenous BDNF no longer facilitated LTP. Neutralizing BDNF activity by TrkB-IgG, however, significantly inhibited tetanus-induced LTP in the adult hippocampus [57].

These experiments are consistent with the studies using BDNF knockout mice and imply that the level of BDNF in the brain is important for tetanus-induced LTP. Application of exogenous BDNF promotes LTP in neonatal hippocampus where the endogenous BDNF level is low, while mutation of BDNF gene or reduction of BDNF activity by TrkB-IgG has the opposite effect in the adult hippocampus where the endogenous BDNF level is high (Fig. 1). Finally, the mechanism by which BDNF regulates hippocampal LTP has been found to be due to a significant enhancement of hippocampal synapses to respond to tetanic stimulation rather than to an alteration of the LTP triggering mechanism. Hippocampal synapses exhibited a severe synaptic depression when stimulated with repetitive, high-frequency stimulation. In hippocampal slices treated with BDNF, however, synaptic depression was significantly attenuated. These results suggest that the BDNF effect on LTP is mediated by an enhancement of the ability of hippocampal synapses to respond to high-frequency, tetanic stimulation (Fig. 2).

Role of GDNF in the development of kidney and enteric nervous system

GDNF was first discovered for its ability to enhance the survival of midbrain dopaminergic neurons in culture [8]. Subsequent studies demonstrated that GDNF also has potent neurotrophic activities for many peripheral neuronal populations [59–61]. However, the major focus of the functional studies of GDNF has been on the midbrain dopaminergic neurons and spinal cord motoneurons, because these neurons are degenerated in patients with neurological disorders such as Parkinson's disease and amyotrophic lateral sclerosis (ALS). The degeneration of the nigrastriatal dopaminergic system induced by mechanical lesions and toxic chemicals such as 1-methyl-4-phenylpyridinium and 6-hydroxydopamine is significantly attenuated by GDNF [14, 15, 62–68]. Moreover, a single administration of GDNF has been shown to rescue the motor deficits in a parkinsonian monkey model [16]. Extensive studies also indicate that GDNF prevents motoneuron cell death both during development and after axotomy in the adult [69–72]. These results suggest that GDNF is an important neurotrophic factor for brainstem dopaminergic neurons and spinal cord motoneurons, and raise the exciting possibility that GDNF may be used as a therapeutic agent for ALS and Parkinson's disease.

Recent characterization of GDNF null mutants has confirmed the role of GDNF in several ganglia of the peripheral nervous system: there is a significant reduction in the neuronal populations of the petrosal and nodose ganglia (40%), superior cervical ganglia (35%), and dorsal root ganglia (23%) in GDNF-deficient mice [73, 74]. However, the GDNF knockout experiments have generated a few surprises regarding GDNF functions during embryonic development [73–75]. First, the midbrain dopaminergic neurons and noradrenergic neurons of the locus ceruleus appear to be normal in the GDNF knockout mice. Despite the report that GDNF is a potent trophic factor for motoneurons so far discovered [70], there is only a 21% reduction of spinal lumbar motor nuclei [73, 74]. These results suggest that GDNF is not a physiological survival factor for these neurons in vivo. Second, the mutant animals do not form permanent kidneys. The homozygote mutants die within 24 h after birth, presumably due to kidney failure. Third, these mice completely lack the enteric nervous system (ENS). Thus the GDNF knockout study has taught us an important lesson: to fully understand the biological function of neurotrophic factors pharmacological experiments should be complemented by molecular genetic studies, and deletion mutations can uncover certain aspects of function which are otherwise difficult to reveal. The GDNF function during normal development in animals, as well as the molecular mechanism of GDNF action at the tissue and cell levels, needs to be understood in much more detail before a rational course of therapy can be charted.

The lack of defects in midbrain dopaminergic neurons in the GDNF knockout mice [73, 74] was unexpected, since this population responds dramatically to the treatment with exogenous GDNF. It is possible that there may be other GDNF-related trophic factors which compensate for the lack of GDNF function in the mutants. Alternatively, GDNF may not be a survival factor for the dopaminergic neurons but can mimic the function of other GDNF-like trophic factors. This is consistent with the

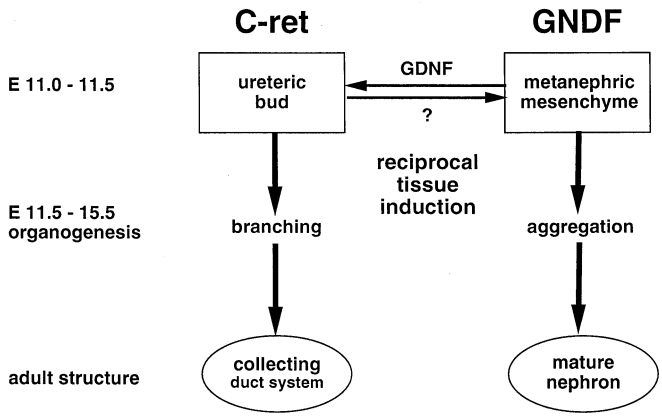

Fig. 3 Role of GDNF during kidney development

recent discovery of a new member in theGDNF family, nutririn. The search for additional GDNF-like molecules is now underway.

The development of the kidney depends on sequential and reciprocal interactions of two primordial tissues, the ureteric bud and the metanephric mesenchyma (Fig. 3) [76]. The ureteric bud induces the metanephric mesenchyma to aggregate and proliferate, leading to the formation of the epithelium of the mature nephron [77]. Meanwhile, the ureteric bud itself is induced by the metanephric mesenchyma to proliferate and branch, ultimately generating the collecting duct system. The role of GDNF in rodent kidney development has in fact been implied by its expression, which coincides with the morphogenesis of the kidney. Metanephric tissues begin to differentiate at embryonic day 11 (E11), and by E13.5 high levels of GDNF expression are detected in condensing metanephric mesenchyma near the tip of ureter bud, the area of the prospective kidney nephrogenic tubules, and maintained through E15.5 (Fig. 3) [78–81]. Soon after epithelization kidney tubules cease to express GDNF. The GDNF knockout studies demonstrate that GDNF is indeed a metanephric mesenchyma-derived factor that induces ureteric bud formation and branching (Fig. 3) [73–75]. Furthermore, the growth or arborization of the ureteric bud of the GDNF mutants is dramatically stimulated by exogenous GDNF in a dose-dependent manner during early stages of kidney morphogenesis [75].

In normal mice in situ hybridization experiments have shown abundant expression of GDNF mRNA in the outer mesenchymal layer of the entire developing gastrointestinal tract [73, 80, 81]. GDNF null mice exhibit a dilation of the proximal intestine and a severe occlusion of the pyloric sphincter, suggesting a defect in those innervations that coordinate control of the gastrointestinal motility. Detailed analysis indicate the complete lack of the neural crest derived enteric nervous system, beginning as early as E12.5 [73–75]. Preliminary experiments have found that GDNF enhances the differentiation and fiber outgrowth of the enteric neurons in a dose-dependent manner (unpublished results). These data indicate that GDNF is an important factor for the proper development of enteric neurons in the digestive tract. The gastrointestinal defect in the GDNF mutant resembles a human genetic disorder, Hirschsprung's disease (HSCR), named after the Danish physician who first described the disorder in 1887 (for review, see [82]. This disease is characterized by absence of intrinsic enteric ganglion cells in the myenteric and submucosal plexuses of the gut, leading to megacolon formation [83, 84]. Interestingly, some autosomal dominant forms of Hirschsprung's disease have been associated with mutations in the human RET locus [85, 86]. Furthermore, mice defective in the c-ret gene have phenotypes almost identical to the GDNF mutants. The c-ret homozygous mutants do not form the ureteric bud or undergo metanephric development and lack ENS neurons [87].

The striking similarity between the c-ret and GDNF knockouts in both the gastrointestinal and the kidney phenotypes suggests that c-ret may mediate GDNF signaling. Indeed, c-ret, which was initially discovered as an orphan receptor tyrosine kinase [88], has recently been found to be a critical component of the receptor signaling system for GDNF [9, 10]. Moreover, expression cloning has recently identified GDNFR-α as the ligand binding subunit of the receptor complex [11, 12]. Biochemical evidence revealed that c-ret is an integral signal transducing component for the GDNF signaling mechanism. GDNF binds with high affinity to GDNFR-α, which in turn recruits c-ret into the ligand-receptor complex and induces tyrosine phosphorylation of c-ret [11, 12]. The c-ret receptor tyrosine kinase is expressed in cell populations that respond to GDNF, including midbrain dopaminergic neurons, motoneurons, ureter bud cells, and the enteric ganglioblasts [89]. Identification of the GDNF signaling pathway will facilitate our understanding of the molecular and cellular mechanisms of GDNF action in diverse systems.

Clinical implications of the novel functions of neurotrophic factors

Recent studies reviewed here indicate that in addition to its classic role in the survival and differentiation of motoneurons and sensory neurons the neurotrophin BDNF may regulate learning and memory, particularly during development. The clinical implications of these findings are twofold. First, one must take this novel BDNF function into consideration when BDNF is considered as a therapeutic agent for the treatment of neurological disorders. For example, a number of studies in vitro and in vivo, including a variety of lesion and toxin models have indicated that BDNF promotes the survival and differentiation of mesencephalic dopaminergic neurons in substantia nigra. When considering a BDNF therapy for Parkinson's disease, it is important to remember that BDNF may also alter the cognitive functions if delivered into the brain, and the side effect may be more severe in children than adults. Currently the only clinical trial for BDNF is on its effect on the motoneuron disease

ALS. Since BDNF cannot cross the blood-brain barrier, muscle injection of BDNF may not interfere with learning and memory and other cognitive functions. Second, regardless of its mechanism, the effect of BDNF on learning and memory also raises the possibility of activating the BDNF/TrkB system as a potential therapeutic strategy for learning disorders in children and adults.

Since its first discovery GDNF has promised to be a therapeutic agent for the treatment of neurodegenerative diseases, particularly Parkinson's disease and motoneuron diseases such as ALS [90, 91]. In addition to the data in rodents, recent experiments have demonstrated that a single administration of GDNF dramatically corrects the motor deficits in a parkinsonian monkey model [16]. These studies have formed the basis for the initiation of a clinical trial to test the therapeutic role of GDNF in Parkinson's diseases. However, the recent work on GDNF knockout mice has revealed an unexpected function of GDNF in the development of the kidneys and the enteric nervous system. Thus caution must be exercised with the clinical use of GDNF. In motoneuron diseases the side effects of systemic or muscle delivery of GDNF on the overgrowth of kidney and ENS must be tested. For treatment of Parkinson's disease further experiments are required to determine whether CNS delivery of GDNF would cause any side effects in the periphery. On the other hand, since severe defects in kidney and ENS development are observed in mice with GDNF mutation, one can envisage the potential therapeutic uses of GDNF both for defects of renal dysgenesis and for Hirschsprung's disease.

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