STIMULATION OF NEUROTROPHIN SYNTHESIS BY 4-METHYLCATECHOL: A PROMISING APPROACH FOR NEUROPROTECTION

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Neurotrophins play a crucial role in the differentiation, maintenance, and survival of various types of peripheral and central neurons. However, the therapeutic use of neurotrophins is limited by their inability to cross the blood-brain barrier and their instability in the bloodstream. One of the promising approaches to utilize neurotrophic actions of these molecules in the therapy of neurodegenerative diseases is the stimulation of neurotrophin synthesis. Here we review the effects of 4-methylcatechol, a nonadrenergic catechol compound, on the synthesis of the neurotrophins nerve growth factor and brain-derived neurotrophic factor in the peripheral and central nervous system. The neuroprotective potential of 4-methylcatechol in animal models of neurodegenerative disorders is discussed, and other agents that enhance neurotrophin synthesis are also mentioned.

INTRODUCTION

The nervous tissue appears to be able to compensate for some injuries by stimulating survival of neurons. However, it can not replace large populations of damaged neurons. This limitation was also proposed by Santiago Ramon y Cajal, who wrote "...in adult centers, the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated. It is for science of the future to change, if possible, this harsh decree." Although emerging evidence suggests in vivo neurogenesis in the adult brain (1), tremendous efforts of neuroscientists are continuing to be focused on the role played by neurotrophic factors in the pathobiology and therapy of both peripheral and central neurodegenerative diseases.

The family of neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, NT-4/5, and NT-6, plays a crucial role in the differentiation, maintenance, and survival of distinct and overlapping neuronal populations within the central and peripheral nervous systems (CNS and PNS, respectively) (2-5). In addition, neurotrophins are related to neuronal plasticity (6,7). Neurotrophins are widely distributed in the CNS, and expressed at the highest level in the hippocampus and cerebral cortex (8-10). Expression of both mRNANGF and mRNABDNF is known to be regulated by glutamate or gamma-aminobutyric acid (GABA) neurotransmission (11-13). And evoked in association with various types of CNS and PNS injuries, such as sciatic nerve lesion (14,15), ischemic and traumatic injuries (11,16), and infusion of kainic acid (17) or 6-hydroxydopamine (18,19) in the brain. These observations suggest an involvement of neurotrophins in the process of neuronal degeneration and regeneration. Indeed, intraventricular administration of BDNF prevents neuronal death of the nigral dopaminergic neurons induced by infusion of neurotoxins (20) or axotomy of nigrostriatal pathway (21,22). Likewise,
administration of NGF or BDNF suppresses neuronal death in the hippocampal pyramidal neurons following transient forebrain ischemia (23,24). Therefore, BDNF in particular, which has much wider action spectrum than NGF, is expected as a therapeutic agent for neurological disorders, such as Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease. However, there are at least two obstacles against therapeutic application of neurotrophins to CNS diseases. First, neurotrophins are macromolecules that cannot pass through the blood-brain barrier (BBB), demonstrating a difficulty to deliver them from the periphery to the CNS. Second, neurotrophins may be rapidly incorporated into the liver due to their cationic charge (25), resulting in a short-term circulation in the bloodstream. Finally, an intraventricular infusion of neurotrophins as therapy involves serious technical and ethical problems. Further, transfection of BDNF gene with viral vectors, and transplantation of the cells engineered with BDNF gene may be promising approaches; a few reports demonstrate their effective protection against dopaminergic neurotoxins (26,27). However, the clinical reality of these applications has not yet been fully established. A promising approach to utilize neurotrophic actions on the therapy is the stimulation of synthesis of neurotrophic factors. In this article, we review the effects of 4-methylcatechol (4MC) on the synthesis of NGF and BDNF in the PNS and CNS, and discuss its potential as a neuroprotective agent for degenerative neurological disorders.

DISCOVERY OF CATECHOL COMPOUNDS THAT STIMULATE NGF SYNTHESIS IN VITRO

In the PNS, NGF is synthesized predominantly in the target tissues of sympathetic neurons, taken up into the axons of these neurons and retrogradely transported into their cell bodies (28,29). NGF levels and mRNA<sub>NGF</sub> expression in the target tissue correlate with the density of sympathetic innervation (30). Moreover, nonneuronal cells, such as astrocytes, fibroblasts, and Schwann cells, are also responsible for NGF synthesis and release in target tissues. Accordingly, cell cultures are tested for NGF production, using a highly sensitive enzyme immunoassay for NGF (31) which enables to detect a small amount of NGF secreted into culture medium of primary fibroblasts (32) and a cell line of L-M fibroblasts (33). During investigation of regulatory mechanism(s) of NGF secretion in these cells, apoprotein stimulatory activity of a series of catechol compounds, including catecholamines, on NGF synthesis is found (33). Examination of the relationship between structure and activity clearly demonstrate that the stimulatory activity is based on the catechol ring and that the potency could be modulated by the side chain structure at the 4-position (34). A series of 4-alkylcatechols and their acetylated derivatives, which affect NGF synthesis without participation of adrenergic receptors, are documented to be potent stimulators of NGF production in vitro (35). In addition to fibroblasts, cultured astrocytes also produced NGF and their NGF production is markedly enhanced by catechol compounds (35,36). Additional active compounds such as propentofylline (37) and idebenone (38), which have chemical structures different from catechols, are found to exert the similar stimulatory activity in cultured astrocytes. The catechol compound 4MC has potent activity, and hence 4MC is used as a model compound that stimulates NGF synthesis.

ENHANCEMENT OF NGF SYNTHESIS IN THE PERIPHERAL NERVOUS SYSTEM

We assessed the action of 4MC on NGF production in rat tissues in vivo. After repeated injection of 4MC, the levels of NGF in heart and submaxillary glands is increased. The most effective dose (10 (ig/kg body weight) was much smaller than expected from the experiments in vitro. Single 4MC administration induces a transient increase in NGF and mRNA<sub>NGF</sub> in the target organs. The increase in NGF level is also observed in the sciatic nerve. These results suggest retrograde axonal transport to the ganglia of the NGF induced in peripheral tissues in a manner similar to that occurs physiologically (39). Figure 1 illustrates 4MC-induced changes in NGF levels. Furthermore, chronic administration of 1,2-diaceotxy propyl benzene, an acetylated, stable form of 4MC analogue, caused significant elevations of substance P levels in dorsal root ganglia and tyrosine hydroxylase activity in superior cervical ganglia of infant rats. These observations suggest that these compounds could stimulate NGF synthesis in vivo, and that the induced NGF is physiologically active on peripheral neurons (39).

STIMULATION OF PERIPHERAL NERVE REGENERATION

Local administration of NGF at the injury site of the sciatic nerve has an influence on subsequent axonal regeneration (40), and NGF synthesis in nonneuronal cells is stimulated in response to sciatic nerve degeneration (41). These observations prompted us to test 4MC for peripheral nerve regeneration in a sciatic nerve-lesioned animal model. The sciatic nerve of adult male Wistar rats was transected and both of the cut ends were inserted into silicone tubes that were subsequently attached to an intervening silicone chamber. The rats were then injected intraperitoneally every day for 2 weeks with 10 J·kg·kg of 4MC. Two weeks after surgery, the density of nonmyelinated axons within the chamber was significantly increased in the 4MC-treated group. Five weeks after surgery, both the number and the diameter of myelinated axons within the chamber of the 4MC-treated group were significantly larger than those of the control group. When the chamber was filled with anti-NGF antibody solution, most of the 4MC effects were blocked. These observations suggest that 4MC stimulates de novo synthesis of NGF and/or NGF-related molecules such as BDNF, resulting in enhanced sprouting and maturation of proximal axons (42). Total number of myelinated axons in the regenerated sciatic...
Figure 1. Schematic representation of a time- or region-specific increase in NGF levels following a single intraperitoneal injection of 4MC. In the heart and submaxillary gland, NGF content increased between 12 and 16 hr, and then decreased to the original level between 20 and 32 hr. The time-course of changes in NGF level in the sciatic nerve, which is one of the routes of NGF transport from the peripheral tissues to the ganglia, certifies axonal transport. Namely, the increase in NGF appears in the peripheral side at 20 hr, and it moves to the central side by 24 hr. The maximal increases at 40 hr in DRG and at 32 hr in SCG occur 16 to 24 hr after those seen in the end organs. These results suggest retrograde axonal transport to the ganglia of the NGF induced in the peripheral tissues in a manner similar to that occurs physiologically.
SUPPRESSION OF PERIPHERAL NEUROPATHIES

Decrease in NGF content in the sciatic nerve (43) and rate of the sciatic nerve regeneration (44) is found in streptozotocin (STZ)-induced diabetic rats. These findings suggest a close relationship between NGF action and sciatic nerve regeneration. Recent work reveals a fall in sciatic motor nerve conduction velocity (MNCV) and a significant reduction of NGF content in the sciatic nerve of STZ-induced diabetic rats (45). 4MC treatment of these rats for 4 weeks, starting from the STZ injection, results in an elevation of NGF content and prevents the reduction of MNCV, but exerts no effect on high glucose levels (45). These results suggest that decreased NGF levels in the sciatic nerve of experimental diabetic rats may be involved in the development of neuropathic process, also reported in human diabetic neuropathy (45a). 4MC treatment, via stimulation of NGF synthesis, may represent a viable neuroprotective strategy for the therapy of diabetic neuropathy (46a for neurotrophins, particularly, NGF in the therapy of human diabetic neuropathy).

The potential efficacy of 4MC on an experimental model of the die-back type of peripheral neuropathy is also examined, using acrylamide monomer (ACR)-induced neuropathy in rats. This neuropathy results in a significant reduction in both MNCV and density of large myelinated fibers. In rats, 4MC, administered intraperitoneally together with ACR, improve clinical signs, and significant increase in NGF content in the sciatic nerves, faster MNCV, and greater myelinated fiber density than in rats given ACR alone (47). These findings suggest that 4MC can prevent the progression of ACR-induced neuropathy, in which decreased NGF levels may be involved.

ENHANCEMENT OF BDNF SYNTHESIS

As described above, the range of 4MC actions extends to effects on NGF-nonresponsive motor and sensory neurons (42,47), suggesting that 4MC could stimulate synthesis of neurotrophic factors other than NGF. Indeed, in cultured rat hippocampal neurons, the secretion of BDNF is increased when 4MC is added, while 4MC does not induce such an effect on NGF, NT-3, or glial cell line-derived neurotrophic factor (48). This suggests a selective action of 4MC on BDNF gene expression in hippocampal neurons, both in vitro and in vivo.

In next experiments, infant rats less than 10 days old, at which time the BBB is not yet fully established (49), are used, so that 4MC might better penetrate into the brain. Dose-dependent enhancement of mRNA expression, estimated by in situ hybridization, is observed in neurons of the whole brain, including the cerebral cortex, hippocampus, thalamus, and cerebellar Purkinje cells (48,50) (Fig. 3). However, the expression of mRNA in the infant rat brain is not detected, irrespective of the period and dosage of 4MC administration. Time-related changes in mRNA and BDNF-like immunoreactivity are chased in the cerebral cortex following a single intraperitoneal injection of 4MC (50) (Fig. 4). mRNA is maximally elevated at 1 h, decreased from 3 h, and recovered to the pretreatment level by 12 h post-4MC injection in layers II/III and V of the cerebral cortex. BDNF-like immunoreactivity is elevated markedly in layer V and slightly in layer II/III 3 h after the inject ion, and the increased levels were sustained even at 12 h in layers II/III and V. Western immunoblot analysis shows that BDNF-like increases time-dependently from 6 to 15 h before recovering to the pretreatment level by 24 h after the 4MC injection. These findings demonstrate that 4MC, penetrating into the BBB, strongly stimulates brain BDNF synthesis in the rat.

INFLUENCES ON CALBINDIN D-28 EXPRESSION

Brain BDNF synthesis induced by 4MC may affect certain neuronal functions. This was evaluated by monitoring the ex-
Stimulation of neurotrophin synthesis

Figure 3. Enhanced expression of mRNA<sup>BDNF</sup> in an infant rat brain following repetitive administration of 4MC. Newborn rats were injected intraperitoneally with vehicle alone, or with 10 or 150 μg of 4MC/kg body weight at 12-hr intervals for 10 days. The rats were anesthetized and cardioperfused with cold 4% paraformaldehyde 4 hr after the final injection of 4MC before brain sections of each group were hybridized with antisense or sense cRNA probe for mRNA<sup>BDNF</sup>. Digoxigenin-labeled cRNA probes were generated with T<sup>7</sup> or SP6 RNA polymerase using pGEM-7Zf(+) plasmid with a full-length mouse BDNF cDNA as a template.

POTENTIAL OF 4MC AS A THERAPEUTIC AGENT

4MC is believed to be incorporated into the cells via a mechanism similar to that for uptake 2 of catecholamines (33). Also, 4MC regulates NGF gene expression via both protein kinase C- and cAMP-independent mechanisms in cultured astrocytes (53). A long-lasting enhancement of c-jun mRNA expression is also caused by 4MC (54), which generates AP-1 proteins that drive NGF gene expression (55). However, AP-1 is not required for the activation of BDNF gene (56). The stimulatory effect on mRNA<sup>BDNF</sup> expression is, so far, reported for agents which increase cAMP levels in astrocytes (57), forlipopolysaccharide in microglia (58), and for glutamate receptor agonists in neurons (12,13,56,59). Although these observations suggest an involvement of c-AMP dependent- and/or Ca<sup>2+</sup>-induced signaling, at present, there are no plausible mechanisms that explain 4MC actions on BDNF gene expression. The most serious problem of 4MC for therapeutic use is a difficulty to cross the BBB of matured brains. It is reported that the BBB is partially destroyed in some neurological disorders, such as multiple sclerosis and Alzheimer's disease (60-62). In fact, repetitive peripheral administration of 4MC enhances mRNA<sup>BDNF</sup> expression in infant rats, in which BBB is not yet fully established. Otherwise, chemical modifications that can deliver 4MC into the brain would be promising for patients with healthy BBB functions. By peripheral administration, Kourounakis et al (63) have succeeded to deliver a substantial level of 4MC esterized with dihydropyridine into the brain, and observed significant elevation of brain NGF content.

Recent investigations have added novel roles of BDNF for CNS, such as facilitation of neural transmission (64-66), synapse formation (67,68), synapse plasticity (69,70), regulation of growth of dendrites and axons (71-73), and also expression of genes acting on brain development (74-76). Furthermore, stimulating environment exerts positive effects on cerebral health via increased BDNF expression (77). These observations demonstrate the importance of BDNF for brain to develop, maintain functions, and protect neurons from various insults, and suggest that drug-induced enhancement of brain BDNF synthesis is profitable for prevention and amelioration of particular degenerative neurological disorders. Specifically, the
Figure 4. Transient increases in the expression of mRNA<sub>BDNF</sub> and BDNF-like immunoreactivity in the cerebral cortex after a single injection of 4MC. A single injection of 4MC in 10 jj.1 was administered intraperitoneally to 7-day-old rats at 150 p.g/kg body weight. The rats were anesthetized and cardioperfused with cold 4% paraformaldehyde at 0, 1, 3 and 12 hr after the 4MC injection. Frozen brain sections of 10 jMn thickness were used for in situ hybridization (upper side) and immunostaining (lower side) experiments.

findings that 4MC can elevate in vivo brain BDNF content and/or mRNA<sub>BDNF</sub> expression should be noticed.

**INVOLVEMENT OF NEUROTROPHIN SYNTHESIS IN PATHOBIOLOGY AND THERAPY OF NEUROLOGICAL DISEASES**

Recent studies suggest that estrogen replacement therapy can reduce the risk and severity of AD-related dementia in post-menopausal women (78-80). Estrogen is shown to protect against ischemic injury (81) and neurotoxic effects of p-amylo-id (81,82), increase synaptic sprouting (83), and enhance the functional status of cholinergic projections to the hippocampus and cortex (84), resembling to the action of NGF and/or BDNF. Indeed, estrogen is proved to stimulate both mRNA<sub>NGF</sub> and mRNA<sub>BDNF</sub> expression in rats in vitro and in vivo (85). It is likely that at least a part of estrogen action is mediated by an enhancement of NGF and/or BDNF production. Further, the cAMP system is involved in antidepressant action (86,87). Chronic administration of antidepressants, including selective serotonin reuptake inhibitor, stimulates the cAMP pathway such as expression of cAMP response element binding protein (CREB)(86). AsBDNFgene can be also regulated by CREB(88), BDNF is likely to be involved in antidepressant actions and the pathophysiology of depression. Indeed, stress decreases mRNA<sub>BDNF</sub> expression (89,90), which may contribute to the atrophy and dysfunction of hippocampal neurons (91,92). In
contrast, antidepressant treatment increases the expression of hippocampal BDNF (93), and thereby reverses the stress-induced neuronal atrophy or protects neurons from further damage. Altogether, upregulation of cAMP and BDNF may be a new target for the development of antidepressant agents (93; 94, this volume of Biomedical Reviews). And, agents other than 4MC that also enhance the synthesis of NGF exert neuroprotective effects in both ischemic brain injury (95-103) and diabetic neuropathy (104,105).

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