Biomedical Reviews 1999; 10:45-54.
Dedicated to Rita Levi-Montalcini
©The Bulgarian-American Center, Varna, Bulgaria
ISSN1310-392X

STIMULATION OF NEUROTROPHIN SYNTHESIS BY4-METHYLCATECHOL: A PROMISING APPROACH FOR NEUROPROTECTION

Shoei Furukawa, Atsumi Nitta, and Yoshiko Furukawa[1]
Laboratory of Molecular Biology, Gifu Pharmaceutical University, Gifu, Japan, and 'AichiBunkyo Women's College, Inazawa, Aichi, Japan

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. ***BiomedRev 1999; 10:****45-54.*

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. Every thing 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 $mRNA^{NGF}$ and $mRNA^{BDNF}$ 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,

Received 5 October 1999 and accepted 1 December 1999.
Correspondence and reprint requests to Dr Shoei Furukawa. Laboratory of Molecular Biology, Gifu Pharmaceutical University,Mitahora-higashi, Gifu502-8585, Japan. E-mail: furukawa@gifu-pu.ac.jp

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 scrolosis, 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 security 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 MGF 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). NGFlevels and $mRNA^{NGF}$ expression in the targettissue 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, apotent stimulatory activity of a series of catechol compounds, including catecholarnines, 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 fromcatechols, 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 $mRNA^{NGF}$ 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-diacetoxy 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-tg/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

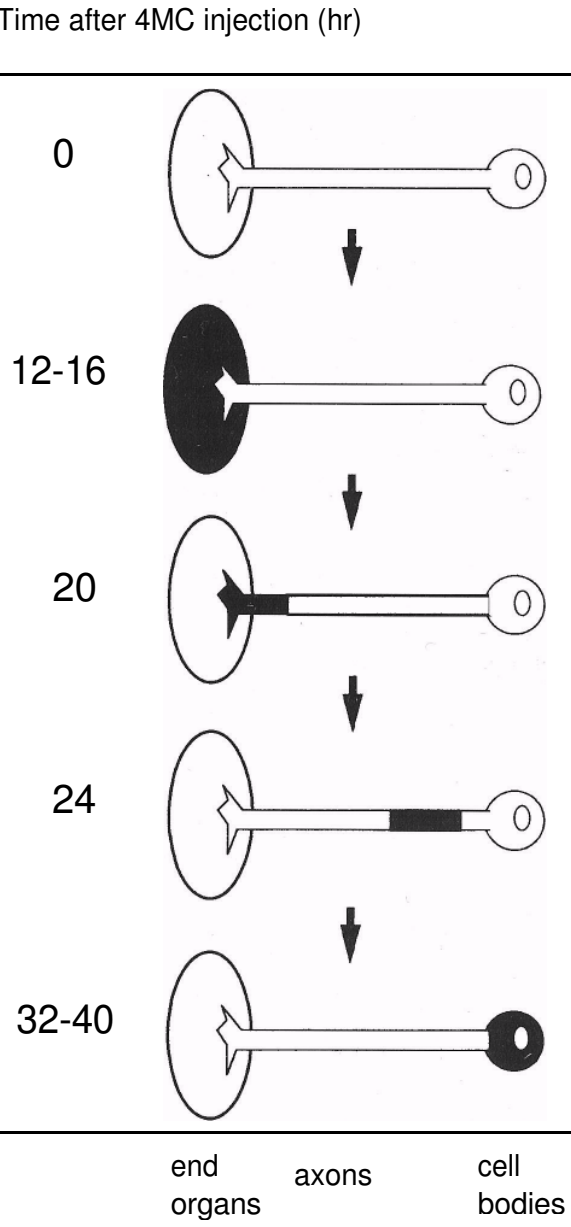

Figure 1. *Schematic representation of a time- or region-specific increase in NGF levels following a single intraperitoneal injection of4MC. In the heart and submaxillary gland, NGF content increased between 12 and 16 hr, and then decreased to the original level between 20 and 32 hr. The time-course 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.*

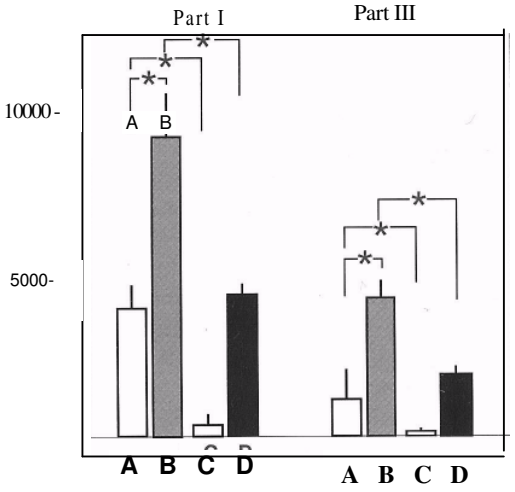

Figure 2. *Total number of regenerated myelinated axons in the proximal portion (part I) and at the midpoint (part III) of the lesioned sciatic nerve of control group (A), 4MC-treated group (B), antibody-applied group (C) and 4MC-treated and antibody-applied group (D) at 5 weeks after the transection. The values are the mean ± SD (n = 6). ^Significance, p < 0.001 (Student's t test).*

nerve of each group is shown in Figure 2.

SUPPRESSION OF PERIPHERAL NEUROPATHIES

Decrease in NGF content in the sciatic nerve (43) and rate of the sciatic nerve regeneration (44)isfoundinstreptozotocin(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 (MNC V) 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 may compensate for the inhibitory effect of STZ on the NGF level in diabetic neuropathy. Further experiments show that the 4MC treatment, started four weeks after STZ injection, result in higher NGF content, faster MNCV, and larger mean diameter of myelinated nerve fibers, compared with untreated rats (46). Taken together these findings demonstrate that 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 ofncu-rotrophic factors other than NGF. Indeed, in cultured rat hip-pocampal 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 day s 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 $mRNA^{BDNF}$ 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 $mRNA^{NGF}$ in the infant rat brain is not detected, irrespective of the period and dosage of 4MC administration. Time-related changes in $mRNA^{BDNF}$and BDNF-like immunoreactivity are chased in the cerebral cortex following a single intraperitoneal injection of 4MC (50) (Fig. 4). $mRNA^{BDNF}$is maximally elevated at 1 h, decreased from 3 h, and recovered to the pretreatment level by 12 hpost-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 injecti on, 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 CALBINDIH D-28 EXPRESSION

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

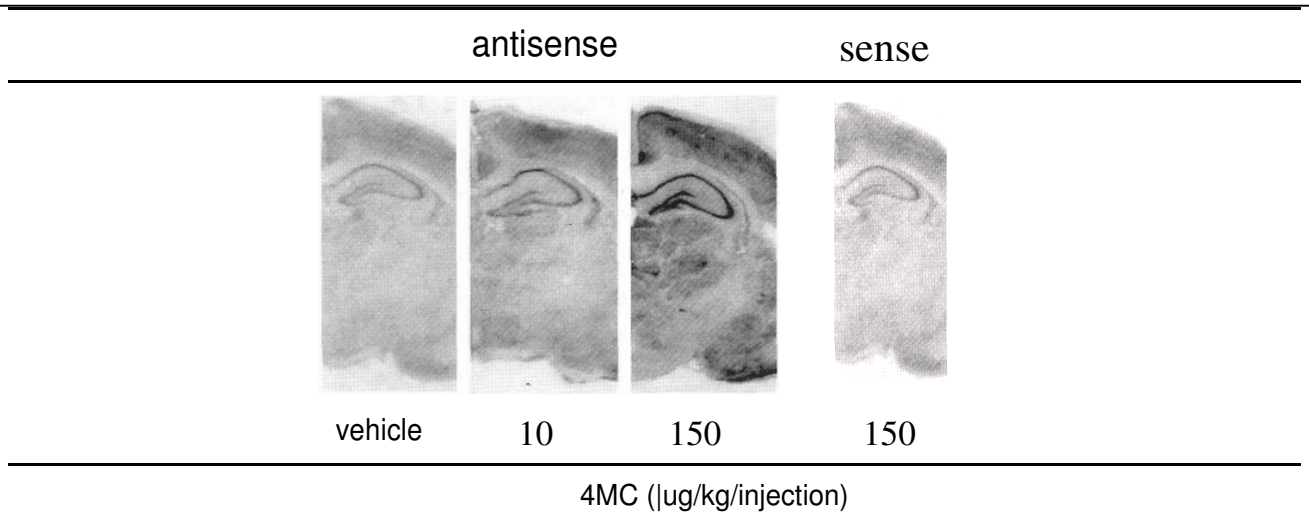

Figure 3. Enhanced expression ofmRNA^{SDNF} in an infant rat brain following repetitive administration of4MC. Newborn rats were injected intraperitoneally with vehicle alone, or with 10 or 150 \ig of4MC/kg body weight at 12-hr intervals for 10 days. The rats were anesthetized and cardioperfused with cold4%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^{BDNF}. Digoxigenin-labeled cRNA probes were generatedwithT? or SP6RNApolymerase using pGEM-7Zf(+)plasmidwithafull-length mouse BDNFcDNAas a template.

pression of calbindin D-28, because it expresses during neuronal maturation (51), and is induced by BDNF in cultured neurons (52). 4MC that was intraperitoneally administered for 10 days to newborn rats elicited significant increases in calbindin D-28 immunoreactivity in dentate granule cells, mossy fibers, and CA3 stratum lucidum of the hippocampus, and certain neuronal populations in the pyriform cortex (50). These findings suggest that subchronic 4MC administration accelerates physiological neuronal differentiation, probably through enhanced BDNF production.

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^{BDNF} 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^{2+}-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^{BDNF} expression in infant rats, in which BBB is not yet fully established. Otherwise, chemical modifications that can deli ver4MC into the brain would be promisingforpatients with heal thy 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

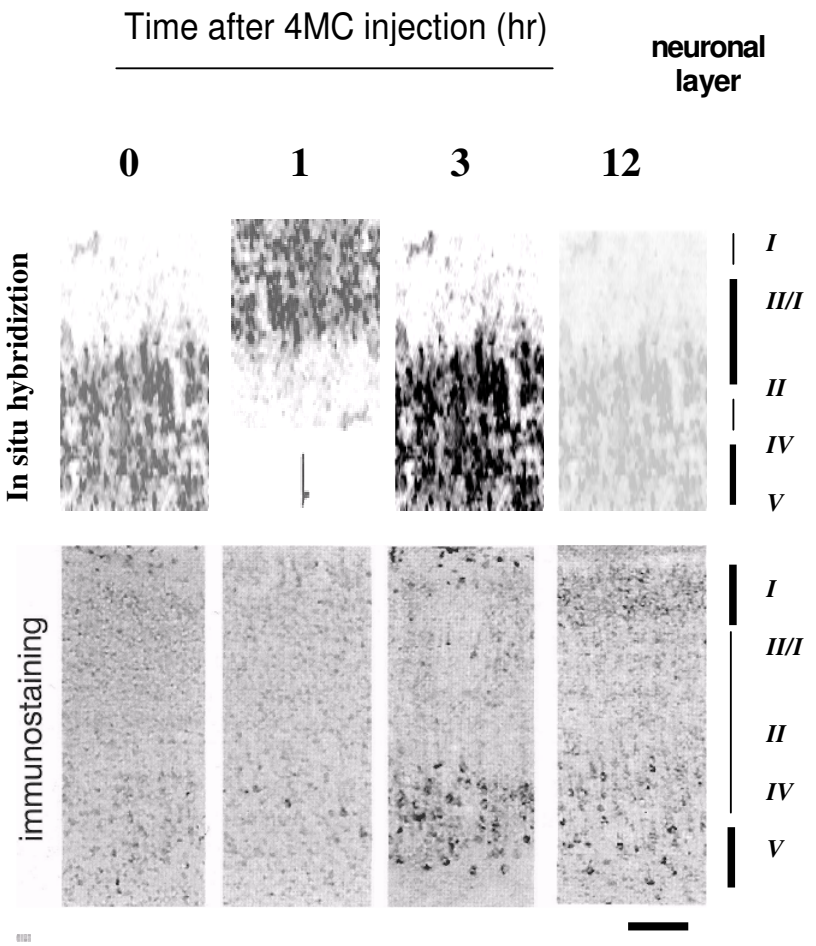

Figure 4. Transient increases in the expression of mRNA^{BDNF} and BDNF-like immunoreactivity in the cereb

ral 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^{BDNF} 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^{NOF} and mRNA^{BDNF} 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). AsBDNFgenecanbealsoregulatedbyCREB(88), BDNF is likely to be involved in antidepressant actions and the pathophysiology of depression. Indeed, stress decreases mRNA^{BDNF} 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 c AMP 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 neuro-protective effects in both ischemic brain injury (95-103) and diabetic neuropathy (104,105).

REFERENCES

1. Doetsch F, Caille I, Lim DA, Carcia-Verdugo JM, Al varez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adultmammalian brain. *Cell* 1999; 97:703-716.
2. Levi-Montalcini R. The history of the discovery of the nerve growth factor. *J Neurobiol* 1993; 24: 893-897.
3. Davies A. The role of neurotrophins in the developing nervous system. *J Neurobiol* 1994; 25: 1334-1348.
4. Snider WD. Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Ce//1994;77:627-638.
5. Lewin GR, Barde Y-A. Physiology of the neurotrophins. *AnnuRevNeurosci 1996; 19:289-317.*
6. Thoenen H. Neurotrophins and neuronal plasticity. Science 1995; 270:593-598.
7. Bonhoeffer T. Neurotrophins and activity-dependent development of the neocortex. *Curr Opin Neurobiol* 1996; 6:119-126.
8. Ernfors P, Wetmore C, Olson L, Persson H. Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. *Neuron* 1990; 5:511-526.
9. Hofer M, Pagliusi R, Hohn A, Leibrock J, Barde Y-A. Regional distribution of brain-derived neurotrophic factor mRNAintheadultmouse brain. *EMBOJ* 1990; 9:2459-2464.
10. Phillips HS, Mains JM, Laramee GR, Rosenthal A, Winslow J W. Widespread expression of B DNF but not NT- 3 by target areas of basal forebrain cholinergic neurons. *Science* 1990; 250:290-294.
11. Lindvall O, Ernfors P, Bengzon J, KokaiaZ, Smith ML, Siesjo *EKetal.* Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 in the adult rat brain following cerebral ischemia and hy-*poglyctm\ccoma.ProcNatlAcadSciUSA* 1992; 89:648-652.
12. ZafraF, HengererB, Leibrock J, Thoenen H, Lindholm D. Activity dependent regulation of BDNF and NGF mRNA in the rat hippocampus is mediated by non-NMDA glutamate receptors.£M5(9/1990; 9:3545-3550.
13. Zafra F, Castren E, Thoenen H, Lindholm D. Interplay between glutamate and y-amino butyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. *ProcNatlAcadSciUSA* 1991;88:10037-10041.
14. Lindholm D, HeumannR, MeyerM, Thoenen H. Interleukin-1 regulates synthesis of nerve growth factor in non-neu-ronal cells of rat sciatic nerve. *Nature* 1987; 330:658-659.
15. Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J Cell Biol 1992; 119:45-54.*
16. HicksRR,NumanS,DhillonHS,PrasadMR, Seroogy KB. Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma. *MolBrainRes* 1997; 48:401-406.
17. BallarinM,ErnforsP,LindeforsN,PerssonH. Hippocampal damage and kainic acid injection induce a rapid increase in mRNA for BDNF and NGF in the rat brain. *Exp Neural* 1991; 114:35-43.
18. NittaA,FurukawaY,HayashiK,HiramatsuM,Kameyama T, HasegawaTef *al.* Denervation of dopaminergic neurons with 6-hydroxydopamine increases nerve growth factor content in rat brain. *Neurosci Lett* 1992; 144: 152-156.
19. Zhou J, Pliego-Rivero B, Bradford HF, SternGM. TheBDNF content of postnatal and adult rat brain: the effects of 6-hydroxydopamine lesions in adult brain. *Brain Res Dev BrainRes* 1996; 97:297-303.
20. TsukaharaT, TakedaM, ShimohamaS, OharaO,Hashimoto N. Effects of brain-derived neurotrophic factor on 1 -methy 1-4-phenyl-1,2,3,6-tetrahydropyridine-induced pakinsonism in monkeys. *Neurosurgeryl995;37:733-739.*
21. Spina MB, Squinto SP, Miller J, Lindsay RM, Hymann C. Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and N-methyl-4-phenylpyridinium ion toxicity: involvement of glutathione system. *JNeurochem* 1992; 59:99-106.
22. Yan Q, Elliott J, Snider WD. Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature* 1992; 360:753-755.
23. Shigeno T, Mima T, Takakura K, Graham DI, Kato G, Hashimoto Y *et al.* Amelioration of delayed neuronal death of the hippocampus by nerve growth factor. *J Neurosci* 1991; 11:2914-2919.
24. Beck T, Lindholm D, Castren E, Wree A. Brain-derived neurotrophic factorprotects against ischemic cell damage in rat hippocampus. *JCereb Blood Flow Metab* 1994; 14:689-692.
25. PardridgeWM, Kang WS,BuciakJL. Transport of human recombinant brain-derived neurotrophic factor (BDNF) through the rat blood-brain barrier in vivo using vector-mediated peptide drug delivery. *Pharm Res* 1994; 11: 738-746.
26. LevivierM, PrzedborskiS, Bancsics C, KangUJ.Intrastriatal implantation of fibroblasts genetically engineered to pro-

duce brain-derived neurotrophic factor prevents degeneration of dopaminergic neurons in a rat model of Parkinson's disease. *J N euros ci* 1995; 15: 7810-7820.

27. FrimDM, UhlerTA, GalpernWR, BealMF, BreakefieldXO, Isacson O. Implanted fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevent 1-methyl-4-phenylpyridinium toxicity to dopaminergic neurons *mtherat.ProcNatlAcadSci USA* 1994;91: 5104-5108.
28. Levi-Montalcini R. The nerve growth factor 35 years later. *Science* 1987;237:1154-1162.
29. Miller FD. Neuronal life or death: how do neurotrophins decide? *Neural Notes* 1998; 3: 3-7.
30. Korsching S, Thoenen H. Nerve growth factor in sympathetic ganglia and corresponding target organs of the rat: correlation with density of sympathetic innervation. *Proc NatlAcadSciUSA* 1983; 80:3513-3516.
31. FurukawaS, Kamo I, AkazawaS, Furukawa Y, SatoyoshiE, Itoh K *et al.* A highly sensitive enzyme immunoassay for mouse P nerve growth factor. *J Neurochem* 1983; 40: 734-744.
32. Furukawa Y, Furukawa S, Satoyoshi E, Hayashi K. Nerve growth factor secreted by mouse heart cells in culture. *JBiol Chem* 1984;259:1259-1264.
33. Furukawa Y, Furukawa S, Satoyoshi E, Hayashi K. Catecholamines induce an increase in nerve growth factor contentinthemediumofmouseL-Mcells. *J BiolChem* 1986; 261:6039-6047.
34. Furukawa Y,FurukawaS,SatoyoshiE, Hayashi K. Aliphatic side chain of catecholamine potentiates stimulatory effect of the catechol part on the synthesis of nerve growth factor. *FEBSLett* 1986; 208:258-262.
35. Furukawa Y, Fukazawa N, Miy ama Y, Hayashi K, Furukawa S. Stimulation of 4-alkylcatechol and their diacetylated derivatives on the synthesis of nerve growth factor. *Biochem Pharmacol* 1990; 40:2337-2342.
36. Furukawa S,Furukawa Y, Satoyoshi E, Hayashi K. Regulation of nerve growth factor synthesis/secretion by catecholamine in cultured mouse astroglial cells. *Biochem BiophysResCommun* 1987; 147:1048-1054.
37. Shinoda I, Furukawa Y, FurukawaS. Stimulation of nerve growth factor synthesis by propentofylline in cultured mouse astroglial cells. *Biochem Pharmacol\990;2>9:*1813-1816.
38. Takeuchi R, Murase K, Furukawa Y, FurukawaS, Hayashi K. Stimulation of nerve growth factor synthesis/secretion by 1,4-benzoquinone and its derivatives in cultured mouse astroglial cells. *FEBSLett* 1990; 261:63-66.
39. Kaechi K, Furukawa Y, Ikegami R, Nakamura N, Ornae F, Hashimoto Y *et al.* Pharmacological induction of physiologically active nerve growth factor in rat peripheral nervous system. *J'PharmacolExp Ther\993;* 264: 321-326.
40. Rich KM, Alexander TD, Pryor JC, Hollowell JP. Nerve growth factor enhances regeneration through silicone chambers. *ExpNeurol\989;* 105:162-170.
41. Heumann R, Korsching S, Bandtlow C, Thoenen H. Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection./Ce//B/o/ 1987; 104: 1623-1631.
42. Kaechi K, Ikegami R, NakamuraN, NakajimaM, Furukawa Y, FurukawaS. 4-methylcatechol, an inducer of NGF synthesis, enhances peripheral nerve regeneration across nerve gaps. *J Pharmacol Exp Ther* 1995; 272:1300-1304.
43. HellwegR, HartungHD. Endogenous levels of nerve growth factor (NGF) are altered in experimental diabetes mellitus: a possible role for NGF in the pathogenesis of diabetic neuropathy. *JNeurosciRes* 1990; 26:258-267.
44. Ekstrom AR,Tomlinson DR. Impaired nerve regeneration in streptozotocin-diabetic rats. Effects of treatment with an aldose reductase inhibitor. *J Neural Sci* 1989; 93:231-237.
45. Hanaoka Y, Ohi T, Furukawa S, Furukawa Y, Hayashi K, Matsukura S. Effect of 4-methylcatechol on sciatic nerve growth factor level and motor nerve conduction velocity in experimental diabetic neuropathic process in rats. *Exp Neural* 1992; 115:292-296.

45a.Anada P, Terenghi G, Warner G, Kopelman P, Williams-Chestnut RE, Sinicropi DV. The role of endogenous nerve growth factor in human diabetic neuropathy. *NatMed* 1996; 2:703-707.

46. Hanaoka Y, Ohi T, Furukawa S, Furukawa Y, Hayashi K, Matsukura S. The therapeutic effects of 4-methylcatechol, a stimulator of endogenous nerve growth factor synthesis, on experimental diabetic neuropathy in rats. *J Neural Sci* 1994; 122:28-32.

46a.Apfel SC. Neurotrophic factors in the therapy of diabetic neuropathy. *AmJMed* 1999; 107 (2B):34S-42S.

47. SaitaK, Ohi T, Hanaoka Y, FurukawaS, Furukawa Y, Hayashi K *et al.* A catechol derivative (4-methylcatechol) accelerates the recovery from experimental acrylamide-induced neuropathy. / *Pharmacol Exp Ther* 1996; 276: 231-237.
48. Nitta A, Ito M, Fukumitsu H, Ohmiya M, Sometani A, Nomoto H *et al.* 4-methylcatechol increases brain-derived neurotrophic factor content and mRNA expression in cultured brain cells and in rat brain in vivo. *J Pharmacol Exp Ther\999;29\:* 1276-1283.
49. KimKS, Wass CA, Cross AS. Blood-brain barrier permeability during the development of experimental bacterial meningitis in the rat. *Exp Neural* 1997; 145:253-257.
50. Fukumitsu H, Sometani A, Ohmiya M, Nitta A, Nomoto H, Furukawa Y *et al.* Induction of a physiologically active brain-derived neurotrophic factor in the infant rat brain by peripheral administration of 4-methylcatcchol. *Neurosci Lett* 1999; 274:115-118.
51. KurobeN, Inaguma Y, Shinohara H, Semba R, Inagaki T, Kato K. Developmental and age-dependent changes of 238-kD calbindin-Dinthe central nervous tissue determined with a sensitive immunoassay method. *J Neurochem*

1992; 58: 128-134.
52. Pappas IS, Parnavelas JG. Neurotrophins and basic fibroblast growth factor induce the differentiation of calbindin-containing neurons in the cerebral cortex. *Exp Neural* 1997; 144:302-314.
53. Furukawa Y, FurukawaS, OmaeF, Awatsuji H, Hayashi K. Alkylcatechols regulate NGF gene expression in astroglial cells via both protein kinase C- and cAMP-independent mechanisms. *JNeurosciRes* 1993; 35:522-529.
54. OmaeF, KatsumataT, SakumaM, Furukawa Y, FurukawaS. Prolonged expression of c-jun proto-oncogene by alkyl-catechol followed by elevation of NGF mRNA in cultured astroglial cells. *JNeurosciRes* 1994; 39:290-297.
55. HengererB, Lindholm D,HeumannR,RutherU, WagnerEF, Thoenen H. Lesion-induced increase in nerve growth factor mRNA is mediated by *c-fos.ProcNatlAcadSci USA* 1990; 87:3899-3903.
56. S ano K, Nanba H, Tabuchi A, Tsuchiya T, Tsuda M. BDNF gene can be activated by Ca^{2}* signals without involvement ofdenovoAP-1 *synthesis. BiochemBiophys Res Commun* 1996;229:788-793.
57. Zafra F, Lindholm D, Castren E, Hartikka J, Thoenen H. Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes. *J Neurosci* 1992; 12: 4793-4799.
58. Miwa T, Furukawa S, Nakajima K, Furukawa Y, Kohsaka S. Lipopolysaccharide enhances expression of brain-derived neurotrophic factor in cultured ratmicroglia. *JNeurosciRes* 1997;50:1023-1029.
59. Castren E, Zafra F, Thoenen H, Lindholm D. Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. *ProcNatlAcad Sci USA* 1992; 89: 9444-' 9448.
60. Cornford EM, Cornford ME. Nutrient transport and the blood-brain barrier in developing animals. *FedProc* 1986; 45:2065-2072.
61. Elovaaral, IcenA, PaloJ, ErkinjunttiT. CSFinAlzheimer's disease. Studies on blood-brain barrier function and intrathecal protein synthesis. *J Neural Sci* 1985; 70: 73-80.
62. Leonard! A, GandolfoC, CaponnettoC, ArataL, Vecchia R. The integrity of the blood-brain barrier in Alzheimer's type and multi-infarct dementia evaluated by the study of albumin and IgG in serum and cerebrospinal fluid. *J Neural* Sdl985;67:253-261.
63. Kourounakis A, Bodor N, Simpkins J. Synthesis and evaluation of brain-derived targeted chemical delivery systems for the neurotrophomodulator 4-methylcatechol. *J PharmPharmacol* 1997; 49:1-9.
64. Kang HJ, Schuman EM. A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science 1996;273:1402-1406.
65. Tongiorgi E, Righi M, Cattaneo A. Activity-dependent dendritic targeting of BDNF and TrkB mRNA in hippocampalneurons. *JNeurosci* 1997; 17:9492-9504.
66. LevineES.-CrozierRA, BlackEB, PlummerMR. Brain-derived neurotrophic factor modulates hippocampal synaptic transmission by increasing N-methyl-D-aspartic acid receptor activity. *ProcNatlAcad Sci USA* 1998;95:10235-10239.
67. CausingCG, GlosterA, AloyzR.BamjiSX, ChangE, Fawcett *letal.* Synaptic innervation density is regulated by neuron-derivedBDNF.TVewron 1997; 18:257-267.
68. VicarioAC,CollinC,McKayRD,SegalM. Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. *J Neurosci* 1998; 18:7256-7271.
69. Korte M, Kang H, Bonhoeffer T, Schuman E. A role for BDNF in the late-phase of hippocampal long-term potentia-*oi\.Neuropharmacology\99?,;* 37:553-559.
70. PattersonSL.GroverLM, SchwartzkroinPA,BothwellM. Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF andNT-3mRNAs.Newro«1992;9:1081-1088.
71. McAllisterAK, KatzLC,LoDC. Neurotrophin regulation of cortical dendritic growth requires activity. *Neuron* 1996; 17: 1057-1064.
72. McAllister AK, Katz LC, Lo DC. Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. *Neuron* 1997; 18: 767-778.
73. Murphy DD, ColeNB, SegalM. Brain-derived neurotrophic factor mediates estradiol-induced dendritic spine formation in hippocampal neurons. *ProcNatlAcadSciUSA* 1998; 98: 11412-11417.
74. Nawa H, Bessho Y, Carnahan J, Nakanishi S, Mizuno K. Regulation of neuropeptide expression in cultured cerebral cortical neurons by brain-derived neurotrophic factor. *J Neurochem\993;60:*772-775.
75. Marty S, Onteniente B. BDNF and NT-4 differentiate two pathways in the modulation of neuropeptide protein levels in postnatal hippocampal interneurons. *Eur J Neurosci* 1999; 11:1647-1656.
76. RingstedtT, LinnarssonS, Wagner J, Lendahl U, Kokaia Z, Arenas E *et al.* BDNF regulates reelin expression and Cajal-Retzius cell development in the cerebral cortex. *Neuron 1998-21:*305-315.
77. Young D, Patricia AL, Paola L, Michael D, During M. Enviromental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nat Med* 1999; 5: 448-453.
78. DelagarzaVW. New drugs for Alzheimer'sdisease. *AmFam Physician* 1998;58:1175-1182.
79. Diaz-Brinton R, Yamazaki RS. Advances and challenges in the prevention and treatment of Alzheimer' s disease. *Pharm Res* 1998; 15:386-398.
80. Schneider LS, Farlow MR, Pogoda JM. Potential role for estrogen replacement in the treatment of Alzheimer's dementia. *Am JMed* 1997; 103:46S-50S.

81. DubalDB, KashonML,PettigrewLC,Ren JM,Finklestein SP, Rau SW *etal.* Estradiol protects againstischemic injury. *JCereb Blood Flow Metab* 1998; 18:1253-1258.
82. Mook-Jung I, Joo I, Sohn S, Kwon HJ, Huh K, Jung MW. Estrogcn blocks neurotoxic effects of (3-amyloid (1-42) and induces neuriteextension on B103 cells. *NeurosciLett* 1997; . 235:101-104.
83. StoneDJ, Rozovsky I, Morgan TE, Anderson CP, FinchCE. Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implications for Alzheimer's disease./Wewrasa 1998; 18:3180-3185.
84. Gibbs RB, Aggarwal P. Estrogen and basal forebrain cholinergic neurons: implications for brain aging and Alzheimer' s disease-related cognitive decline. *HormBehav* 1998; 34:98-111.
85. Simpkins JW, Green PS, Gridley KE, SinghM, deFiebreNC, Rajakumar G. Role of estrogen replacement therapy in memory enhancement and the prevention of neuronal loss associated with Alzheimer's disease. *AmJMed* 1997; 103:19S-25S.
86. NibuyaM, NestlerEJ, DumanRS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. / *Neurosd* 1996; 16:2365-2372.
87. Duman RS. Novel therapeutic approaches beyond the serotonin receptor. *Biol Psychiatry* 1998; 44:324-335.
88. Finkbeiner S, Tavazoie SF, Maloratsky A, Jacobs KM, Harris KM, GreenbergME. CREB: a major mediator of neuronal neurotrophin responses. *Neuron* 1997; 19:1031-1047.
89. Smith MA, Makino S, Kvetnansky R, Post RM. Effects of stress on neurotrophic factor expression in the rat brain. *Ann N YAcadSci* 1995; 771:234-239.
90. Smith MA, Makino S, Kvetnansky R, Post RM. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *JNeurosci* 1995; 15:1768-1777.
91. Stein-Behrens B, MattsonMP, ChangI, YehM, Sapolsky R. Stress exacerbates neuron loss and cytoskeletal pathology in the hippocampus. *JNeurosci* 1994; 14: 5373-5380.
92. NittaA,OhmiyaM,SometaniA,ItohM,NomotoH,Furukawa Y *etal.* Brain-derived neurotrophic factor prevents neuronal cell death induced by corticosterone. *JNeurosci Res* 1999; 57:227-235.
93. Altar CA. Neurotrophins and depression. *Trends Pharmacol* Sc/1999;20:59-61.
94. Shintani F. Cytokines and neurotrophins in psychiatric disorders. *BiomedRev* 1999; 10:69-73.
95. Fisher M, Jones S, Sacco RL. Prophylactic neuroprotection for cerebral ischemia. *Stroke* 1994;25:1075-1080.
96. Semkova I, Schilling M, Henrich-Noack P, Rami A, Krieglstein J. Clenbuterol protects mouse cerebral cortex and rat hippocampus from ischemic damage and attenuates glutamate neurotoxicity in cultured hippocampal neurons by induction of NGF. *Brain Res* 1996; 717:44-54.
97. Zhu Y, CulmseeC, Semkova I, Krieglstein J. Stimulation of p2-adrenoceptors inhibits appoptosis in rat brain after transient forebrain ischemia. *J Cereb Blood Flow Metab* 1998; 18:1032-1039.
98. CulmseeC, StummRK,SchaferMK,WeiheE, Krieglstein J. Clenbuterol induces growth factor mRNA, activates astrocytes, and protects rat brain tissue against ischemic *damage. EurJ Pharmacol* 1999; 379:33-45.
99. Semkova I, Wolz P, Schilling M, Krieglstein J. Selegiline enhances NGF synthesis and protects central nervous system neurons from excitotoxic and ischemic damage. *Em JPharmacol* 1996; 315:19-30.
100. Abu-Ray a S, Blaugrund E, Trembo vler V, Shilderman-B loch E, Shohami E, Lazarovici P. Rasagiline, a monoamine oxidase-B inhibitor, protects NGF differentiated PC 12 cells against oxygen-glucose deprivation. *JNeurosci Res* 1999; 58:456-463.
101. Li P, Matsunaga K, Yamamoto K, Yoshikawa R, Kawashima K, Ohizumi Y. Nardosinone, a novel enhancer of nerve growth factor in neurite outgrowth from PC12D cells. *NeurosciLett* 1999; 273: 53-56.
102. Yamata K, Nitta A, Hasegawa T, Fuji K, Hiramatsu M, Kameyama T *et al.* Orally active NGF synthesis stimulators: potential therapeutic agents in Alzheimer's disease. *Behav Brain Res* 1997; 83: 117-122.
103. Nishio T, Sunohara N, Furukawa S, Akiguchi I, Kudo Y. Repeated injections of nicergoline increase the nerve growth factor level in the aged rat brain. *JpnJ Pharmacol* 1998; 76:321-323.
104. Ohi T, Saita K, Furukawa S, Ohta M, Hayashi K, Matsukura S. Therapeutic effects of aldose reductase inhibitor on experimental diabetic neuropathy through synthesis/secretion of nerve growth factor. *ExpNeurol* 1998; 151:215-220.
105. Riaz S, MalcangioM, Miller M, Tomlinson DR. A vitamin D(3) derivative (CB1093) induces nerve growth factor and prevents neurotrophic deficits in streptozotocin-diabetic *mts.Diabetologial999;42:*1308-1313.