Molecular Neurobiology

ISSN0893-7648/98/16(1): 79–95/$12.25

Role of MAP Kinase in Neurons

Kohji Fukunaga* and Eishichi Miyamoto

Department of Pharmacology, Kumamoto University School of Medicine, Kumamoto 860, Japan

Abstract

Extracellular stimuli such as neurotransmitters, neurotrophins, and growth factors in the brain regulate critical cellular events, including synaptic transmission, neuronal plasticity, morphological differentiation and survival. Although many such stimuli trigger Ser/Thr-kinase and tyrosine-kinase cascades, the extracellular signal-regulated kinases, ERK1 and ERK2, prototypic members of the mitogen-activated protein (MAP) kinase family, are most attractive candidates among protein kinases that mediate morphological differentiation and promote survival in neurons. ERK1 and ERK2 are abundant in the central nervous system (CNS) and are activated during various physiological and pathological events such as brain ischemia and epilepsy. In cultured hippocampal neurons, simulation of glutamate receptors can activate ERK signaling, for which elevation of intracellular Ca^{2+} is required. In addition, brain-derived neurotrophic factor and growth factors also induce the ERK signaling and here, receptor-coupled tyrosine kinase activation has an association. We describe herein intracellular cascades of ERK signaling through neurotransmitters and neurotrophic factors. Putative functional implications of ERK and other MAP-kinase family members in the central nervous system are give attention.

Index Entries: MAP kinase; central nervous system; hippocampus; glutamate receptor; BDNF; calmodulin-dependent protein kinase; stress-activated protein kinase; synaptic plasticity; apoptosis.

Introduction

Mitogen-activated protein (MAP) kinase, also known as extracellular signal-regulated kinase (ERK), is a member of a family of serine/threonine protein kinases and has important roles in the regulation of cell growth and differentiation in response to stimulation with various growth factors. Effects of these growth factors are mediated by activation of cell-surface receptors with intrinsic protein-tyrosine kinase activity. The activation cascade of MAP kinase by stimulation with growth factors has been well documented (Blenis, 1993; Crews and Erikson, 1993; Davis, 1993; Nishida and Gotoh, 1993). The common pathway leading from the receptor tyrosine

*Author to whom all correspondence and reprint requests should be addressed.

kinase involves a small GTP-binding protein $p21^{ras}$ (RAS), which activates Raf-1, the c-raf-1 protooncogene product. Raf-1 phosphorylates MAP kinase/ERK kinase (MEK), which in turn phosphorylates and activates MAP kinase. In addition, numerous neurotransmitters and hormones also activate MAP kinase through stimulation of either of two classes of receptors, G protein-coupled receptors or ligand-gated ion channel-coupled receptors. Increase in intracellular Ca^{2+} is required to activate MAP kinase, as downstream signals from both kinds of receptor. The signaling of Ca^{2+}-dependent activation of MAP kinase is not fully understood.

The genes of MAP kinase are highly expressed in the CNS (Thomas and Hunt, 1993) and proteins of both ERK1 and ERK2 are widely but differently expressed in various regions of the rat brain (Ortiz et al., 1995). Accumulating evidence suggests physiological and pathological roles for MAP kinase in the CNS. MAP kinase in the rat brain was activated following electroconvulsive shock (Baraban et al., 1993), generalized seizure activity (Gass et al., 1993), or transient brain ischemia (Campos-González and Kindy, 1992). In the present article, we directed attention to regulation of MAP kinase activity by stimulation with neurotransmitters, neurotrophins, growth factors, or cytokines in the brain cells. Functional implications of the enzyme in the CNS are given attention.

Expression in the Central Nervous System

Three rat ERKs, ERK1, ERK2, and ERK3, have been purified and cloned (Boulton et al., 1991), and molecular and biochemical studies of MAP kinases have established that two homologous proteins, ERK1 and ERK2, correspond to the MAP-1 and MAP-2 kinase isozymes, respectively. Northern-blot analysis revealed that high levels of ERK1 and ERK2 mRNA are present in the nervous system of adult rats, in contrast to ERK3 mRNA, which is expressed at high levels early during development (Boulton et al., 1991). *In situ* hybridization histochemistry using specific oligonucleotide probes against ERK1 and ERK2 isoforms revealed the precise localization of these isoforms in the CNS of adult rats (Thomas and Hunt, 1993). The signal for ERK2 mRNA was more intense in many regions, including the cerebral cortex, olfactory bulb, hippocampus, amygdala, basal ganglia (except the globus pallidus and endopeduncular nucleus), basal nucleus, thalamus, hypothalamus, brain stem nuclei, cerebellum, and neurons in the spinal cord. In contrast, hybridization signals for ERK1 were relatively weak and were observed in restricted regions, in comparison to ERK2, including the olfactory bulb, cortex, regions of the hippocampus, amygdala, nucleus basalis of Maynert, substantia nigra, some hypothalamic and brain stem nuclei, and cerebellum, as well as neurons of the spinal cord (Thomas and Hunt, 1995). In the hippocampus, ERK1 was highly expressed in the dentate gyrus and was practically nil in the CA1 region, in contrast to expression of ERK2 in all regions of the hippocampus. In both cultured hippocampal neurons and cortical astrocytes, both ERK1 and ERK2 with molecular masses of 44 and 42 kDa were detected by immunoblotting analysis with antibodies prepared for the C-terminal peptide of ERK1, which recognized both ERK isoforms (Baraban et al., 1993; Kurino et al., 1995).

Expression of the upstream kinases of MAP kinase has been noted in the brain. MEK-1 and -2 are direct upstream kinases of MAP kinase and are derived from different genes. Northern-blot analysis indicates that MEK-2 is expressed at low levels in mouse adult brain and at higher levels in neonatal brain (Brott et al., 1993). This is in contrast to the high levels of MEK-1 expressed in the brain. In addition, a number of MEK-activating kinases have been reported, most notably c-Raf-1 and B-Raf. B-Raf is the most abundant Raf in the brain (Storm et al., 1990; Barnier et al., 1995). Consistent with these observations, the MEK-1 activating kinase was copurifed with B-raf in the bovine brain (Catling et al., 1994).

Activation of MAP Kinase by Stimulation of Glutamate Receptors

MAP kinase was activated following electroconvulsive shock (Baraban et al., 1993; Stratton et al., 1991) or generalized seizure activity (Gass et al., 1993) as well as transient brain ischemia (Campos-González and Kindy, 1992; Hu and Wieloch et al., 1994). These observations suggest that stimulation of glutamate receptors and/or depolarization results in activation of MAP kinase during these pathological events. Indeed, Bading and Greenberg (1991) reported that stimulation of the NMDA receptor leads to tyrosine phosphorylation of MAP kinase in cultured hippocampal neurons. In cultured cortical neurons, stimulation of the metabotropic glutamate and the kainate receptors, but not the NMDA receptor activated the 42-kDa MAP kinase. A burst of spontaneous synaptic activity in cultured cortical neurons was associated with activation of MAP kinase (Fiore et al., 1993). Treatment with a combination of tetrodotoxin, a voltage-dependent sodium-channel blocker and MK-801, a specific NMDA-receptor blocker, largely prevented MAP kinase activity as well as burst of spontaneous synaptic activity in cultured cortical neurons (Fiore et al., 1993).

Activation of MAP kinase by stimulation of glutamate receptors was evident in cultured hippocampal neurons (Kurino et al., 1995). Hippocampal neurons of 7–8 d in culture were stimulated with a short exposure to NMDA. After incubation for indicated times, the cells were frozen in liquid N_2. The MAP kinase activity was determined by in-gel kinase assay containing myelin basic protein as a substrate by SDS-PAGE. As shown in Fig. 1A, treatment with NMDA stimulated 42-kDa MAP kinase activity as well as the Ca^{2+}-independent form of CaM kinase II, as determined by SDS-PAGE. In the in-gel kinase assay, an increase of 400% in autonomous CaM kinase II (Ca^{2+}-independent activity) was evident after short exposure to NMDA and the activity reverted to near basal levels within 10 min (Fig. 1B). Likewise, a tran-

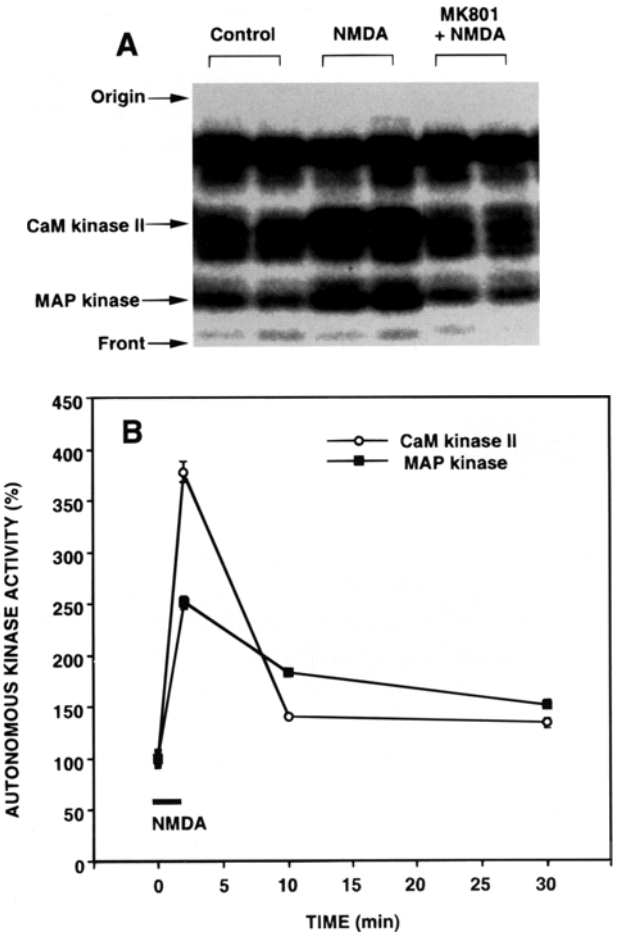

Fig. 1. Time course of MAP kinase and CaM kinase II activation by NMDA treatment in cultured rat hippocampal neurons. **(A)** In-gel kinase assay for MAP kinase and CaM kinase II. Hippocampal neurons of 7–8 d in culture were preincubated with Krebs-Ringer HEPES buffer (KRH) and exposed for 2 min to 100 μ*M* NMDA. After removal of NMDA by changing the medium, the cells were frozen at the indicated time. Cell extracts were prepared and subjected to SDS-PAGE containing myelin basic protein to assay autonomous CaM kinase II and MAP kinase. An autoradiograph shows the NMDA-induced activation of CaM kinase II and MAP kinase. The positions of the enzymes are indicated by arrows. **(B)** Time course of NMDA-induced activation of CaM kinase II and MAP kinase. The relative CaM kinase II and MAP kinase activities were measured using a Bio-Imaging analyzer (BA100 Fujifilm, Tokyo, Japan). The activity is expressed as a percentage of the control (without NMDA) at zero-time. Data are mean ± SE values (n = 6).

sient increase in MAP kinase was also found in the same gel. The NMDA-induced MAP kinase activation was totally prevented by inclusion of AP5 or MK-801 in the incubation medium. NMDA is a potent agonist used to induce MAP-kinase activation (Kurino et al., 1995). In addition to the NMDA receptor, the metabotropic-glutamate receptor, but not AMPA/kainate receptor is involved in glutamate-induced activation of MAP kinase (Kurino et al., 1995). This is in contrast to the observation of cultured cortical neurons in which the AMPA/kainate receptor was mainly involved in MAP kinase activation (Fiore et al., 1993). The signaling cascade of MAP-kinase activation through glutamate receptors was also investigated in the hippocampal neurons (Kurino et al., 1995). Because the glutamate-induced MAP-kinase activation was largely prevented in the presence of protein kinase C (PKC) inhibitor or prolonged incubation with phorbol ester to downregulate PKC (Fig. 2), PKC seemed to be mainly involved in MAP-kinase activation through glutamate receptors. However, a significant level of MAP-kinase activation through glutamate receptors remained after inhibition of PKC, but was totally abolished by removal of extracellular Ca^{2+}. In addition, ionomycin- induced MAP-kinase activation was independent of the PKC pathway. Interestingly, the ionomycin-induced MAP-kinase activation was abolished by inclusion of calmidazolium, a calmodulin antagonist as well as by KN93, a CaM kinase II inhibitor, in the incubation medium (Fukunaga et al., unpublished observation). We tested the effect of KN93 on MAP-kinase activation through various Ca^{2+}-dependent pathways. As shown in Fig. 3, KN93 has a small but significant inhibitory effect on the glutamate-induced MAP-kinase activation. The NMDA- and ionomycin- induced MAP- kinase activation was largely prevented by treatment with KN93, and Bay K8644 (an L-type voltage-dependent Ca^{2+} channel agonist)-induced MAP-kinase activation was abolished by the treatment. These results suggest that the pathways between the glutamate receptor and L-type voltage-dependent Ca^{2+} channel differ

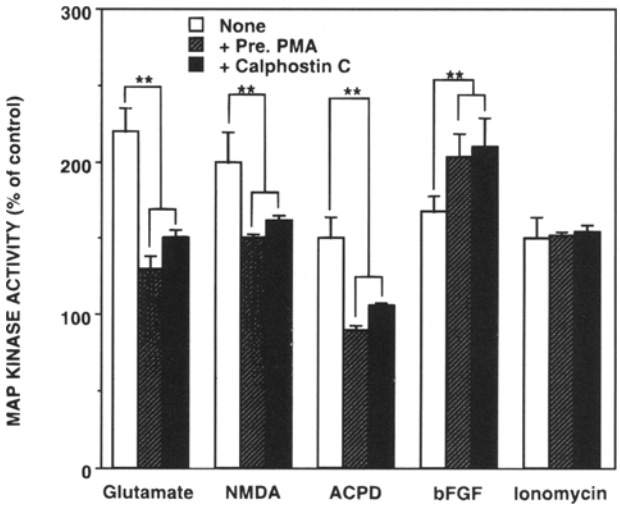

Fig. 2. Effects of protein kinase C downregulation and calphostin C on glutamate-induced activation of MAP kinase in cultured rat hippocampal neurons. For protein kinase C downregulation, hippocampal neurons of 7–8 d in culture were pretreated with 100 n*M* for 16 h (Pre. PMA) as indicated. The cells were then preincubated at 37°C for 30 min in Ca^{2+}-free KRH buffer and incubated for 10 min with 10 μ*M* glutamate, 100 μ*M* NMDA, 300 μ*M* ACPD, 10 ng/mL bFGF, or 5 μ*M* ionomycin in KRH buffer. 100 n*M* calphostin C was added during the last 10 min of preincubation and incubation with test agents. MAP-kinase activities in cell extracts were measured by in-gel kinase assay. Data are mean ± SE values ($n = 6$) and are expressed as percentages of the activity without glutamate, NMDA, ACPD, bFGF, or ionomycin, respectively. Changes in MAP-kinase activity were statistically significant: $^{**}p < 0.01$. From Kurino et al. (1995), with modifications.

with respect to the effects of KN93. Although the mechanism is unknown, the observations strongly indicate that calmodulin and CaM kinases are implicated in the Ca^{2+}-dependent and PKC-independent MAP-kinase activation through glutamate receptors and through the voltage-dependent Ca^{2+} channel. Finkbeiner and Greenberg (1996) proposed that the Ca^{2+}-sensitive Ras guanine nucleotide-releasing factor (Ras-GRF) is a candidate mediating Ca^{2+}-dependent Ras activation. Furthermore, the involvement of c-Raf-1 was not evident in case

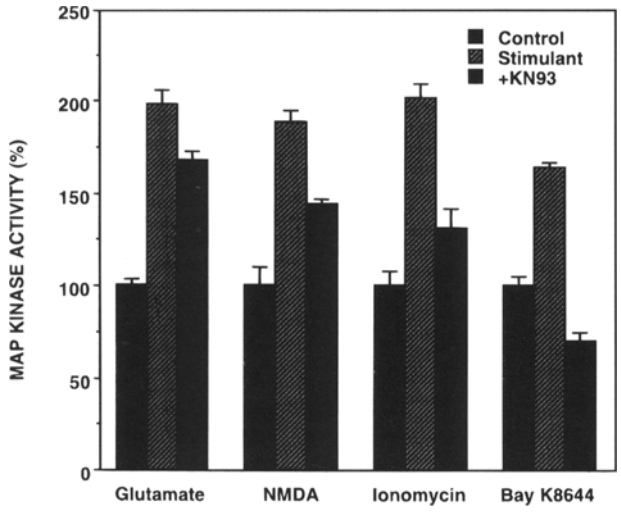

Fig. 3. Effects of KN93 on diverse Ca^{2+}-dependent MAP-kinase activation in cultured rat hippocampal neurons. Hippocampal neurons of 7–8 d in culture were preincubated for 30 min in the presence or absence of 10 μ*M* KN93. The cells were then incubated for 10 min with or without stimulants, including glutamate (10 μ*M*), NMDA (100 μ*M*), ionomycin (5 μ*M*) and Bay K8644 (5 μ*M*). MAP-kinase activities in cell extracts were measured by the in-gel kinase assay as shown in Fig. 1. From Fukunaga et al., unpublished data.

of glutamate-induced MAP kinase activation because of a lack of increased *in situ* phosphorylation of c-Raf-1 (Kurino et al., 1995). In contrast, increased phosphorylation of c-Raf-1 was noted following treatment of the hippocampal neurons with bFGF. The involvement of B-Raf should be tested in case of glutamate-induced activation, since the high expression and the association of B-Raf with MEK in the brain have been documented (Catling et al., 1994).

Activation of MAP Kinase by Stimulation of Neurotrophic and Growth-Factor Receptors

MAP-kinase activation by nerve-growth factor (NGF) has a central role in morphological differentiation of PC12 cells and sympathetic neurons (Marshall, 1995; Robbins et al., 1992; Cowley et al., 1994; Wood et al., 1992). Likewise, multiple neurotrophic factors regulate the development of the nervous system. Among neurotrophic factors, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5 have been well characterized and were seen to have survival-promoting activity in certain types of neurons. For example, BDNF promotes survival and/or differentiation in rat septal cholinergic neurons, dopaminergic neurons in the substantia nigra, GABAergic neurons of the basal forebrain, and rat retinal ganglion cells (Alderson et al., 1990; Johnson et al., 1986; Hyman et al., 1991; Knusel et al., 1991). On the other hand, NT-3 exhibited neurotrophic effects on only neural crest- and nodose ganglion-derived sensory neurons (Knusel et al., 1991; Maisonpierre et al., 1990). In electrophysiological studies, application of BDNF, NT-3, or NT-4/5 potentiates glutamatergic synaptic transmission in cultured hippocampal neurons (Lessmann et al., 1994; Levine et al., 1995) as well as in hippocampal slices of adult rats (Kang and Schuman, 1995). A significant impairment of long-term potentiation (LTP) was observed in hippocampal slices of BDNF-knockout mice (Korte et al., 1995). Consistent with these observations, TrkB and TrkC, tyrosine kinase-coupled receptors specifically recognized by BDNF and NT-3, respectively, are highly expressed in the adult mouse brain (Klein et al., 1989, 1990a, b; Lamballe et al., 1994). Expression of BDNF is upregulated following LTP induction in rat hippocampal slices (Castren et al., 1993; Patterson et al., 1992). Taken together, these observations suggest important roles of neurotrophins in neuronal plasticity in the adult brain as well as developmental plasticity during synaptogenesis in the fetal brain. In both cases, the neurotrophins seem to act as specific retrograde messengers modulating synaptic transmission.

Extensive studies have been focused on intracellular signal transduction of neurotrophins to elucidate physiological functions in neuronal plasticity. Since MAP-kinase activa-

tion through the TrkA receptor, a tyrosine kinase-coupled receptor for nerve-growth factor (NGF) has been well documented concerning survival and morphological differentiation of PC12 cells, the central function of MAP kinase through TrkB and TrkC receptors is understood to be a signaling cascade to elicit modulating effects on neuronal plasticity. Marsh et al. (1993, 1996) reported a persistent activation of MAP kinase with exposure of BDNF to cultured hippocampal neurons. The BDNF-induced MAP kinase activation was also evident in cultured cortical astrocytes (Roback et al., 1995). In both neural cells, induction of c-Fos was closely associated with MAP-kinase activation by stimulation with BDNF. On the other hand, the TrkC receptor for NT-3 was highly expressed in pyramidal neurons but not in astrocytes (Marsh et al., 1996). The extent of the MAP-kinase activation by NT-3 was comparable to that with BDNF in hippocampal pyramidal neurons. Although BDNF and NT-3 have been known to promote neuronal survival on different types of neurons, the neurotrophins do not seem to promote survival of hippocampal pyramidal neurons (Marsh et al., 1996). In addition, BDNF had no apparent effect on mitotic activity in cortical glial cells, as determined by ^{3}H-thymidine incorporation under serum-free conditions. Taken together, BDNF-induced MAP kinase activation may not initiate a cascade that leads to survival promotion and mitogenesis, but may be involved in the modulation of synaptic transmission as a retrograde messenger in hippocampal LTP. This is consistent with the observation of an increased level of BDNF mRNA following the LTP induction (Castren et al., 1993; Patterson et al., 1992).

Application of exogenous neurotrophins such as NGF and BDNF has been seen to increase release of acetylcholine and glutamate in synaptosomal preparations of rat hippocampal neurons (Knipper et al., 1994a,b) and increase dopamine release in cell cultures of rat mesencephalic neurons (Blochl and Sirrenberg, 1996). In cultured hippocampal or cortical neurons, both neurotrophins stimulated phosphorylation of synapsin I, which is localized in presynaptic sites and is associated with synaptic vesicles and microfilaments (Knipper et al., 1994b; Jovanovic et al., 1996). In the case of cortical neurons, Jovanovic et al. (1996) clearly demonstrated that BDNF could stimulate the phosphorylation of synapsin I at sites for MAP kinase by using antibodies that recognize the phosphorylation site of synapsin I by MAP kinase. The phosphorylation by MAP kinase significantly reduced the potential of synapsin I to polymerize G-action and form the bundle of the actin filaments. The phosphorylation site for MAP kinase exists in both head and tail regions of synapsin I and just precedes the sites for CaM kinase II in the tail region. The MAP kinase-induced phosphorylation may account for acute effects on synaptic transmission in the cholinergic and glutamatergic neurons.

In addition to neurotrophins, bFGF and EGF have been seen to activate MAP kinase, in turn promote survival of various types of neurons, and increase the mitogenic activity of brain astrocytes. In cultured rat hippocampal neurons, bFGF promoted the bifurcation and growth of axonal branches without affecting the elongation rate of primary axons (Aoyagi et al., 1994), and resulted in increased complexity of axonal trees. Stimulation of the bFGF receptor associated with tyrosine kinase is coupled to activation of Ras and phospholipase Cγ, which induces activation of MAP kinase and PKC, respectively. Since the promotion of neurite branching activity by bFGF was not blocked by a PKC inhibitor and treatment with PMA, a PKC activator had no effect on the branching. MAP kinase may be involved in the morphological differentiation in cultured neurons. Similar to the neurotrophins, bFGF could modulate synaptic transmission by acting on GABAergic neurotransmission rather than glutamatergic neurotransmission (Tanaka et al., 1996). The molecular mechanisms underlying the synaptic potentiation by bFGF remain to be investigated.

In cultured cortical astrocytes and C6 glioma, bFGF produced a long-lasting increase in MAP-kinase activation (Tournier et al., 1994;

Kurino et al., 1996) and in turn promoted proliferation. The long-lasting increase in MAP-kinase activation was associated with translocation of the enzyme from the cytosol to the nucleus and/or increases in the perinucleus (Kurino et al., 1996) (Fig. 4). Traverse et al. (1992) suggested that the sustained activation of MAP kinase with nerve-growth factor might be required for translocation of the kinase to the nucleus and neuronal differentiation in PC12 cells. However, the transient activation with epidermal growth factor (EGF) was associated with no nuclear translocation of MAP kinase and only potentiated proliferation. Treatment with dibutyryl cyclic AMP or isoproterenol completely abolished the bFGF-induced MAP kinase activity (Kurino et al., 1996) as well as translocation into the nucleus as shown in Fig. 4. Similarly, the bFGF-induced stimulation of ^{3}H-thymidine uptake into astrocytes was abolished by treatment with dibutyryl cyclic AMP or isoproterenol (Kurino et al., 1996). Burgering et al. (1993) and Cook and McCormick (1993) reported that potentiation of DNA synthesis and activation of MAP kinase by stimulation with EGF in Rat 1 fibroblasts were inhibited by cyclic AMP accumulation that interfered in the MAP-kinase signaling cascade at the downstream site of Ras and the upstream site of Raf-1. Similarly, the bFGF-induced Raf-1 phosphorylation was inhibited by treatment with dibutyryl cyclic AMP in cultured astrocytes (Kurino et al., 1996). Häfner et al. (1994) reported that cyclic AMP-dependent protein kinase phosphorylated Raf-1 with concomitant inhibition of the activity. However, the precise molecular mechanisms of the inhibition of growth factor-induced MAP-kinase activation by cyclic AMP are not clear. In brain astrocytes, the cyclic AMP signaling cascade has been seen to promote differentiation rather than proliferation (Fahrig and Sommermeyer, 1993). Treatment of cultured astrocytes with dibutyryl cyclic AMP resulted in a morphological transformation from a flat, polygonal phenotype to a stellate-like cell shape. The differentiation by cyclic AMP elevation in cortical astrocytes was associated with increases in gene expression of different trophic factors and cytokines such as FGF-2 and interleukin-6 (Norris et al., 1994; Riva et al., 1996).

Activation of MAP kinase by the neurotrophins and growth factors has a common pathway leading from the receptor-tyrosine kinase that activates Ras, Raf-1 kinase, and MEKs, and in turn activates MAP kinase. Neurotrophins and growth factors are involved in morphological differentiation such as neurite branching and promote the cell survival. It is now questionable if both functions are elicited by activation of MAP kinase and whether target proteins for the enzyme locate in the neurons.

Activation of MAP Kinase by Stimulation of G-Protein-Coupled Receptors

Activation of MAP kinase through stimulation of neurotransmitter receptors that are coupled with heterotrimeric GTP-binding proteins is clearly evident in neuronal cells. MAP-kinase activation through the acetylcholine muscarinic M2 receptor coupled to G_i was found to be associated with Ras and Raf activation and was insensitive to genistein, a tyrosine-kinase inhibitor (Winitz et al., 1993). MAP-kinase activation mechanisms, through G_i- and G_q-coupled receptors, were further investigated in COS-7 cells that were transiently expressed by alpha-2A- and 1B-adrenergic receptors and muscarinic M1 receptor (Hawes et al., 1995; van et al., 1995). MAP kinase activation through G_i-coupled alpha-2A receptor was mediated by $\beta\gamma$ subunits of G_i and blocked by expression of dominant-negative $p21^{ras}$ as well as by dominant-negative Raf-1. This pathway was sensitive to a tyrosine-kinase inhibitor but was insensitive to PKC depletion by downregulation. In contrast, MAP-kinase activation through G_q-coupled alpha-1B and M1 receptors was not mediated by $\beta\gamma$ subunits and was not blocked by dominant-negative $p21^{ras}$ and tyrosine-kinase inhibitors. However, the

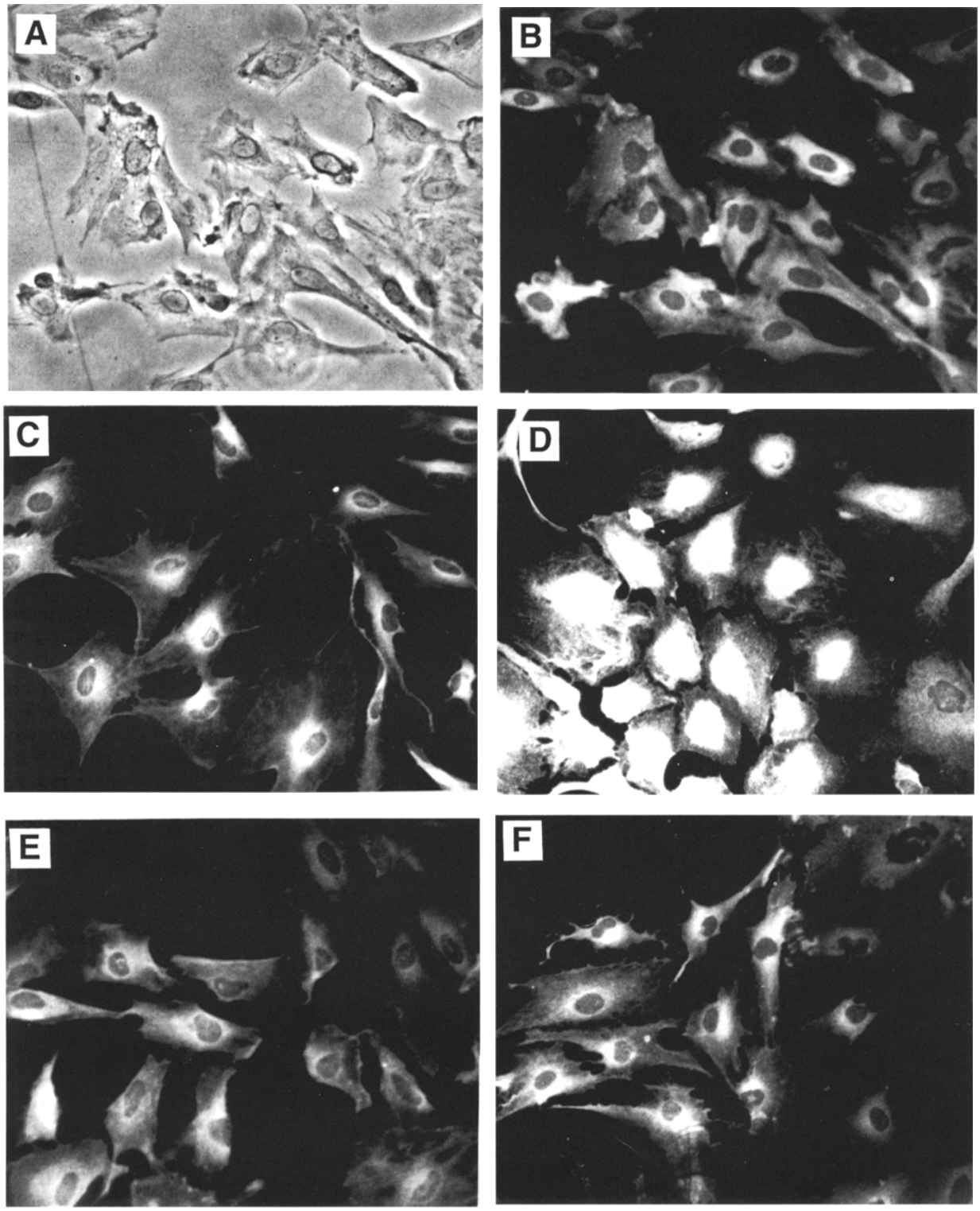

Fig. 4. Translocation of MAP kinase into the nuclei following treatments with bFGF and inhibition of this translocation by dbcAMP and isoproterenol. Indirect immunofluorescent localization of MAP kinase was investigated in cultured rat cortical astrocytes. The astrocytes were incubated for 1 h with no addition (**A** and **B**), 1 m dbc AMP (**C**), 10 ng/mL bFGF (**D**), 10 ng/mL bFGF plus 1 m*M* dbcAMP (**E**), or 10 ng/mL bFGF plus 10 μ*M* isproterenol (**F**) in serum-free culture medium. The cells were fixed in cold methanol, followed by treatment with 0.05% Triton X-100. Fixed cells were incubated with normal rabbit IgG of the rabbit anti-rat MAP-kinase R2 antibody (erk1-CT) (Upstate Biotechnology Inc., New York, NY) and processed to indirect immunofluorescence. The cells were examined by phase-contrast (**A**) and immunofluorescence (**B–F**). 200 X. From Kurino et al. (1996).

MAP-kinase activation was blocked by expression of dominant-negative Raf-1 and PKC depletion. Extensive studies using COS-7 cells with the expressed M2-muscarinic receptor demonstrated that the MAP kinase signaling pathway through βγ subunits was mediated through phosphoinositide 3-kinaseγ (PI3Kγ) and required a tyrosine kinase, probably Src-family kinase, as a downstream kinase (Lopez et al., 1997). Shc, Grab2, Sos, Ras, Raf-1, and MEK underlied in the signaling from the tyrosine kinase to MAP kinase activation, as a common pathway as seen in cases of growth-factor-receptor signaling. Although the PKC-dependent cascade in the activation of MAP kinase through G_q-coupled receptors is not fully understood, activation of Raf-1 through direct phosphorylation by PKC is possibly involved in the PKC-dependent pathway as described (Kolch et al., 1993; Carroll and May, 1994).

The heterogeneity of MAP-kinase signaling through G protein-coupled receptor also remains to be determined. Platelet-activating factor (PAF)-induced activation of MAP kinase is partly prevented by treatment with pertussis toxin (PTX), suggesting that it is G_i-coupled signal, but is not associated with $p21^{ras}$ activation (Honda et al., 1994). In cultured hippocampal neurons, PAF-induced MAP-kinase activation was weakly inhibited by PTX treatment but was abolished by a PKC inhibitor (Fukunaga et al., 1995). This would suggest that PAF-induced MAP-kinase activation in hippocampal neurons is mediated by G_q rather than G_i. Interestingly, in cultured neurons, stimulation of the PAF-receptor potentiated phosphorylation of synapsin I, probably because of activation of MAP kinase (Fukunaga et al., 1995) and in turn stimulated spontaneous excitatory synaptic activity through the AMPA receptor-ion channel (Tokutomi et al., 1997). These observations are consistent with those of BDNF-induced synapsin I phosphorylation and synaptic potentiation, as described above.

In addition to MAP-kinase activation through Gi- and Gq-coupled receptors, stimulation of Gs-coupled receptor may also activate MAP kinase in some cell types, including PC12 cells (Vossler et al., 1997). Accumulation of cyclic AMP does not antagonize the activation of MAP kinases by growth factors but activates MAP kinases (Frodin et al., 1994; Young et al., 1994). Potential role of Rap1, a member of small GTP-binding proteins from the Ras family, has been documented in vivo in the cAMP-mediated activation of B-Raf and in turn, MAP kinases. This is consistent with an observation that B-Raf can be activated by Rap1 in vitro (Ohtsuka et al., 1996).

Functional Implication of MAP Kinase in Physiological and Pathological Events in CNS

Initially, MAP kinase was identified as an insulin-stimulated serine/threonine kinase that phosphorylates microtubule-associated protein 2 (MAP2) and myelin basic protein. In PC12 cells, further characterization of MAP kinase stimulated by treatment with NGF and FGF was performed by using MAP2 as substrate. The *in situ* phosphorylation of MAP2 by MAP kinase was regulated by synaptic activity in both neonatal and adult hippocampal neurons (Quinlan and Halpain, 1996). Although the physiological relevance of MAP kinase-induced phosphorylation of cytoskeletal components such as MAP2 and synapsin I in the organization of the synapse has not been established in the developing nervous system, the phosphorylation of neuronal cytoskeletons are possibly involved in the neurotrophic factor-induced synaptic potentiation in the hippocampus (Lessmann et al., 1994; Levin et al., 1995; Kang and Schuman, 1995). The MAP kinase pathway may also function to regulate synaptic transmission through long-term changes in protein synthesis and gene expression at the nucleus. The stimulation of glutamate-, BDNF-, NT-3-, bFGF-, and PAF-receptors elicits an increase in immediate-early genes such as c-fos and zif-268 in cultured hippocampal neurons (Marsh et al., 1993, 1996; Bading et al., 1993; Ferhat et al., 1993; Bazan and

Allan, 1996). These immediate-early genes are expressed after various physiological and pathological events such as long-term potentiation, seizure, and brain ischemia. A central role of MAP kinase in the expression of the immediate-early genes has been well characterized. The signaling cascade of c-fos expression through glutamate receptors, especially the NMDA receptor, was extensively studied by Greenberg and colleagues (Bading et al., 1993; Miranti et al., 1995; Xia et al., 1996). The NMDA receptor mainly contributes to c-fos gene expression by glutamate stimulation. Elevation of Ca^{2+} through the NMDA receptor stimulates protein-tyrosine kinases including Src-family kinases and protein-tyrosine kinase 2 (PYK2) and in turn potentiates phosphorylation of Shc. The Shc/ Grab2/Sos complex stimulates Ras GDP/GTP-exchanging activity. The potential role of Ras-GRF (Ras-guanine-nucleotide-releasing factor), a member of GDP/GTP exchange factors was found to be involved in the Ca^{2+}-dependent pathway of Ras activation (Farnsworth et al., 1995). Ras-GRF may be related to the Ca^{2+}-dependent Ras activation by stimulation of L-type voltage-dependent Ca^{2+} channel by depolarization. Following the activation of Ras, activation of Raf-1/MEKK and MAP kinase seems to be a common pathway in different stimuli through BDNF, glutamate receptors and L-type voltage-dependent Ca^{2+} channel. However, the signaling cascades underlying c-fos-gene expression differ between NMDA receptor- and depolarization-induced activation, as summarized in Fig. 5. The NMDA-receptor-induced c-fos expression was mediated by transcription factors, including serum- response factor (SRF) and Elk-1, a member of the ternary-complex factors (TCFs). The phosphorylation of Elk-1 was directly mediated by MAP kinase, which in turn bound to serum response element in the promoter region of c-fos gene in combination with SRF as a dimer (Xia et al., 1996). Janknecht and Nordheim (1996) reported that cAMP-dependent protein-kinase can phosphorylate CBP, which binds to CREB and cooperatively potentiates the transcriptional activity of CREB. The regulation of CBP by MAP kinase may also be underlying in the potentiation of c-fos gene expression in cooperation with Elk-1 phosphorylation by MAP kinase (Janknecht and Nordheim, 1996). In contrast, c-fos expression through L-type Ca^{2+}-channel activation was mediated through CaM kinase II and/or CaM kinase IV. CREB is a target transcription factor for CaM kinases and activates c-fos-gene expression by binding to the cyclic AMP-responsive element (CRE) in its promoter region. As shown in Fig. 3, MAP-kinase activation through the L-type Ca^{2+}-channel was totally dependent on activation of CaM kinases because of inhibition by KN93, but the glutamate-induced activation was less well understood. Involvement of CaM kinase IV in MAP-kinase activation was reported by Enslen et al. (1996). In NG108 cells, the transient expression of CaM kinase IV increased activity of members of the MAP-kinase family, including ERK family kinase, c-Jun N-terminal kinase (JNK), and p38 MAP kinase. The mechanism of activation may be functional not only in neuronal cells but also in nonneuronal cells such as smooth muscle cells (Muthalif et al., 1996). Thus, in the depolarization-induced c-fos-gene expression, involvement of the MAP kinase/Elk-1 pathway through the voltage- dependent Ca^{2+} channel remains to be determined, because depolarization stimulates Ras activation through Ras-GRF (Farnsworth et al., 1995) and the depolarization-induced MAP-kinase activation is abolished by KN93 as described above.

In case of bFGF-induced MAP-kinase activation, the activated MAP kinase is translocated into the nucleus where many of the physiological targets for the MAP kinase are located. These substrates may include transcription factors such as c-Myc, c-Jun, and C/EBP beta (NF-IL6) that are known to be regulated by MAP-kinase phosphorylation. Further studies are needed to define the functional roles of transcription factors and their regulation by MAP-kinase phosphorylation in neuronal plasticity.

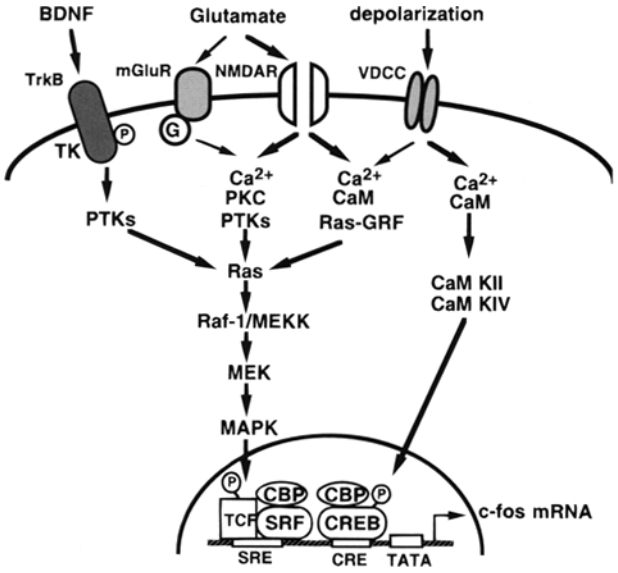

Fig. 5. Signaling pathways for activation of c-fos gene following stimulation with BDNF, glutamate, and depolarization. In cultured hippocampal neurons, stimulation with BDNF, glutamate, or depolarization activates the c-fos gene, through different pathways. The Ras/Raf-1/MAP-kinase pathway mainly contributes to activation of the c-fos gene by stimulation with glutamate and BDNF. In upstream pathways between receptor and Ras activation, the signaling from BDNF receptor, TrkB, has a common pathway with other growth factor receptors containing tyrosine kinase. The diversity of signaling through glutamate receptors to activate Ras has been documented. Both PKC-dependent and Ca^{2+}/calmodulin-dependent pathways have been proposed. Both pathways may share common regulators such as TCF and CBP to activate the c-fos gene. In contrast, stimulation by depolarization may induce the c-fos gene through activation of Ca^{2+}/calmodulin-dependent protein kinases which in turn phosphorylate CREB family proteins. VDCC, voltage-dependent calcium channel; TK, tyrosine kinase; PKC, protein kinase C; CaM, calmodulin; PTKs, protein tyrosine kinases; Ras-GRF, Ras-guanine nucleotide releasing factor; CaM KII and KIV, Ca^{2+}/calmodulin-dependent protein kinases II and IV; CBP, CREB-binding protein; TCF, ternary complex factor; SRF, serum response factor; CREB, cyclic AMP responsive element-binding protein.

Although MAP-kinase activation in pathological events in the brain has been documented, the target molecules of MAP kinase following the activation are not well understood. Phosphorylation of cytosolic phospholipase A2 (cPLA2) is mainly catalyzed by p42 and p44 MAP kinase (ERK1 and ERK2) as well as p38 MAP kinase and results in translocation of the enzyme to membranes and an increase in the intrinsic activity of the enzyme (Durstin et al., 1994; Kan et al., 1996; Kramer et al., 1996; Lin et al., 1993; Sa et al., 1995). cPLA2 is activated and/or induced during brain ischemia (Bazan et al., 1993; Lazarewicz et al., 1992; Owada et al., 1994), an observation consistent with activation of MAP kinase during brain ischemia (Campos-González and Kindy, 1992; Hu et al., 1994). However, activation of cPLA2 dose not always correlate with phosphorylation by MAP kinases (Kramer et al., 1996). The involvement of MAP kinase in phosphorylation of cPLA2 should be investigated during states of brain ischemia. Another possible target for MAP kinase is tau, a microtubule-associated protein, in the brain. Tau is a component of microtubules in soma and axons of neurons and a major component of paired helical filaments (PHFs) observed in brains of patients with Alzheimer's disease. The pathological tau is different from normal tau in its phosphorylation state, in which many Ser-Pro and Thr-Pro motifs in tau protein are highly phosphorylated (Pelech, 1995). Several studies supported the idea that MAP kinase as well as glycogen synthase kinase-3 is implicated in the phosphorylation of tau to produce PHFs (Drewes et al., 1992; Lu et al., 1993; Mandelkow et al., 1992). How the abnormally phosphorylated tau accumulates in PHFs in brains of subjects with Alzheimer's disease has yet to be clarified.

The potential role of MAP kinase in the promotion of neuronal survival seems functional during development of the brain as well as in neurodegenerative disorders. Various neurotrophic and growth factors promote survival as well as morphological differentiation in specific cell types in the brain ERK1 and ERK2, a subgroup of MAP kinase family, were mainly activated by trophic factors and seemed to me-

diate their neurotrophic actions. In addition, other subgroups of MAP-kinase family, c-Jun N-terminal kinase (JNK), and p38 MAP kinase family have been cloned and characterized. Both JNK and p38 MAP kinase have been considered to participate in cellular responses to environmental stresses, inflammatory cytokines, and apoptotic agents rather than mitogenic stimuli. JNK (also named stress-activated protein kinase, SAPK) and p38 MAP kinase are activated by tumor-necrosis factor (TNF), anisomycin, UV light, DNA-damaging agents, interleukin-1, endotoxin, osmotic stress, and heat shock. However, both kinase families have distinct upstream activators and different substrate specificities for transcription factors as target molecules c-Jun, a transcription factor, is a substrate of the JNK-signaling pathway, ATF2 is a target for p38 MAP kinase, and ELK-1 is phosphorylated by all three groups of MAP kinase family. Stimulation of cells with anisomycin and UV radiation induces c-fos and c-jun gene through potentiation of JNK-mediated phosphorylation of Elk-1 and of c-Jun or ATF2, respectively (Hazzalin et al., 1996). The induction of c-Jun is closely related to apoptosis in diverse cell types. Indeed, in case of Alzheimer's disease, the immunoreactivity for c-Jun is elevated in association with the pathological state of neurons (Cotman and Anderson, 1995; Anderson et al., 1994). Beta-amyloid-induced apoptosis was seen to be associated with sustained induction of c-Jun in cultured hippocampal neurons (Anderson et al., 1995). Furthermore, Xia et al. (1995) proposed that JNK and p38 MAP kinase-signaling pathways are implicated in neuronal programmed cell death. In differentiated PC12 cells, NGF withdrawal resulted in apoptosis, which is preceded by an increase in the p38 MAP-kinase activity. Reversibly, activation of ERK signaling by treatment with NGF could eliminate the apoptosis. These opposing effects of ERK and JNK/p38 MAP-kinase signaling were evident in cultured fetal neurons (Heidenreich and Kummer, 1996). The ERK signaling by stimulation with insulin inhibited p38 MAP-kinase activity in cultured neurons. Activation of MAP-kinase phosphatase may be underlying in the insulin-induced p38 MAP-kinase inhibition. This notion is consistent with findings that MAP-kinase phosphatases 1 and 2 (MKP1 and MKP2), dual-specific protein phosphatases, dephosphorylate and inactivate JNK1 and JNK2 that are expressed in COS7 cells in combination with MKP1 or MKP2. Furthermore, in PC12 cells, the UV-stimulated JNK activity is significantly reduced by pretreatment with NGF (Hirsch and Stork, 1997). The expression of MKP1 was reported to be stimulated following limbic epilepsy in the rat brain (Brondello et al., 1997). Another dual-specific phosphatase, PAC-1, was also transcriptionally induced in the rat brain following forebrain ischemia (Wiessner, 1995). Furthermore, the ERK signaling cascade may be directly underlying in the expression of MKP1 and MKP2 (Brondello et al., 1997). Taken together, the cross-talk between ERK signaling and JNK/p38 MAP-kinase signaling has a central role in apoptosis during synaptic elimination and in neurodegenerative disorder. To understand the molecular basis of apoptosis in both neuronal events, the precise signaling cascade of JNK/p38 MAP-kinase activation following apoptotic stimuli has to be established.

In the present article, attention was directed to activation of the MAP-kinase family following diverse stimuli to the brain. Acute effects of neurotrophic and growth factors as well as cytokines such as PAF on the synaptic transmission should be emphasized. MAP kinase is directly implicated in the modulation of synaptic transmission. Although several target proteins such as synapsin I and MAP2 have been proposed regarding substrate for MAP kinase in synapses, further studies are needed to define molecular mechanisms. In addition, MAP kinase activation through the NMDA receptor followed by induction of c-Fos is involved in the gene expression of synaptic components associated with LTP in the hippocampus and account for the long-lasting modification of synaptic transmission. It is an important observation that each of ERKs, JNKs, and p38 MAP kinase has preferential substrates such as NF-IL6, c-Jun, and ATF2, re-

spectively, in the nuclei. On the other hand, some transcription factors such as Elk-1 and CREB/ATF1 serve as a common target for ERKs and p38 MAP kinase (Tan et al., 1996). Taken together, these MAP-kinase family members may have respective specific functions related to survival, neural differentiation, and apoptosis but also have common functions such as neural plasticity, including stress-induced adaptations in the brain.

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