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Mitogen-Activated Protein Kinases and Cerebral Ischemia

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Abstract

Mitogen-activated protein kinases (MAPKs) have crucial roles in signal transduction from the cell surface to the nucleus and regulate cell death and survival. Recent papers support the hypothesis that neuronal apoptosis and cerebral ischemia induce the robust activation of MAPK cascades. Although extracellular signal-regulated kinases pathways promote cell survival and proliferation, and c-Jun N-terminal protein kinases/p38 pathways induce apoptosis in general, the roles of MAPK cascades in neuronal death and survival seem to be complicated and altered by the type of cells and the magnitude and timing of insults. Some specific inhibitors of MAPK cascades provide important information in clarifying the roles of each molecule in neuronal death and survival, but the results are still controversial. Further studies are necessary to elucidate the activated signal transduction upstream and downstream of the cascades in cerebral ischemia, and to define the crosstalk between the cascades and other signaling pathways, before MAPK cascades can be candidate molecules in the treatment of cerebral ischemia.

Index Entries: MAP kinase; cerebral ischemia; neuron; apoptosis; ERK; JNK; p38.

Introduction

Stresses on cells activate intracellular second-messenger systems, including several protein kinases and phosphatases. Mitogen-activated protein kinases (MAPKs), which are characterized as Pro-directed Ser-Thr-protein kinases, have recently been a focus of intensive study, because they are evolutionarily well-conserved across all eukaryotes, and are proven to play several important roles on signal transduction from the cell surface to the nucleus (*1–4;* Fig. 1). The fundamental property of MAPKs is to require the simultaneous phosphorylation of both Thr and Tyr residues to be active. MAPKs are divided in three families, and are distinguished by the nature of the amino acid located between the two phosphorylated residues in the activation loop: the extracellular signal regulated kinases (ERKs) contain the sequence Thr-

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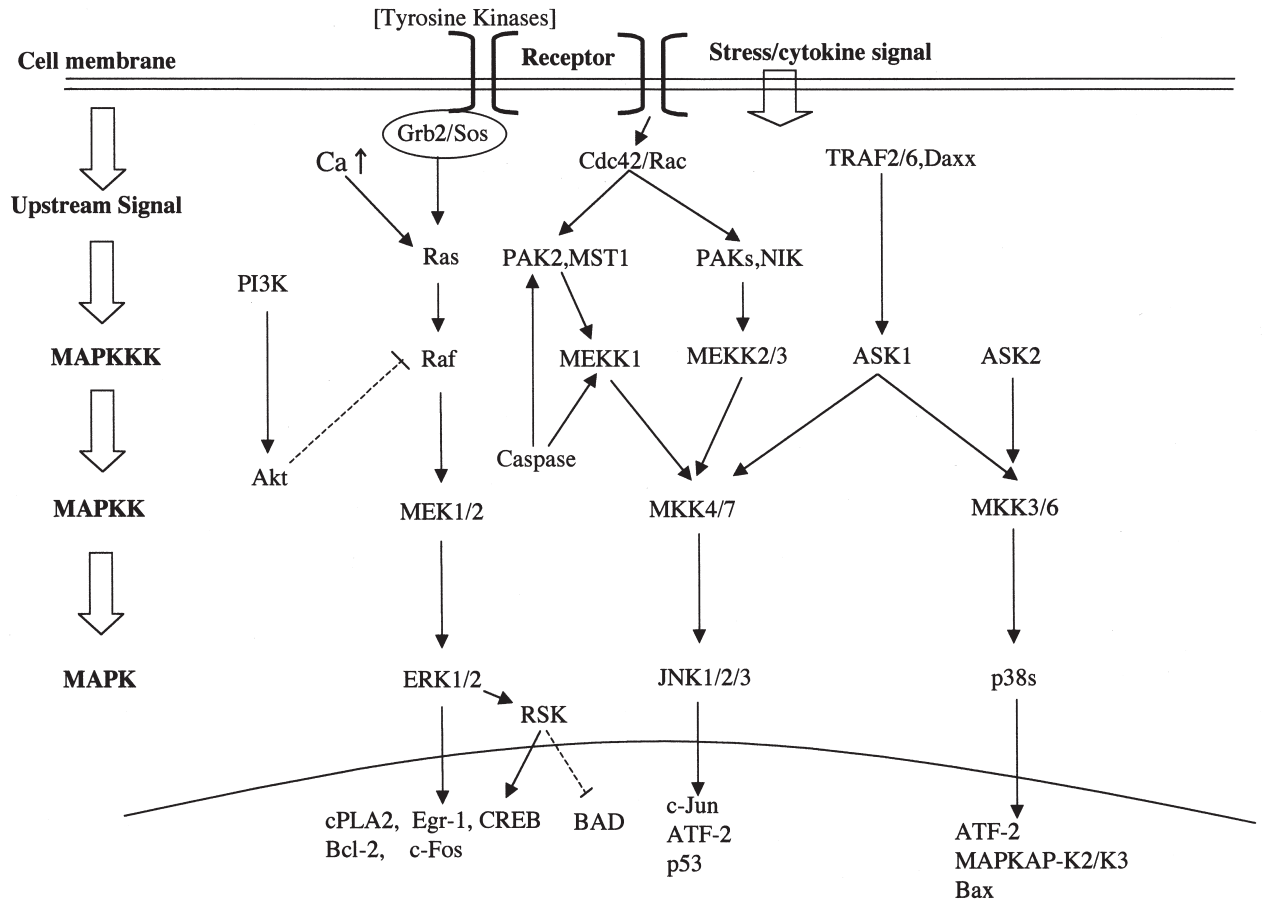

Fig. 1. Signal transductions in MAPK cascades.

Glu-Tyr, and the c-Jun-N-terminal kinases (JNKs) and p38 MAPKs contain the sequence Thr-Pro-Tyr and the sequence Thr-Gly-Tyr, respectively. On activation by phosphorylation on Thr and Tyr residues, MAPKs phosphorylate several important intracellular enzymes and transcription factors.

The ERKs are activated in response to growth factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3–5, basic fibroblast growth factor, and epidermal growth factor, and also to oxidative stress, intracellular calcium increase, and glutamate receptor stimulation. The activation of ERKs links to immediate-early gene induction and hyperphosphorylation of Elk-1 and cyclic adenosine monophosphate (cAMP)/Cal-responsive element-binding protein (CREB). The role of ERKs in cell growth and proliferation is well-established. On the other hand, JNKs and p38 MAPKs are activated in response to a variety of cellular stresses, such as changes in osmolarity and metabolism, DNA damage, heat shock, or inflammatory cytokines, and modulate several transcriptional factors, such as transcriptional factor activator protein-1 and nuclear factor-κ B. Phosphorylation of JNKs results in c-Jun induction, and phosphorylation of p38 MAPKs results in MAPK-activated protein 2 and acti-

vating transcription factor 2 (ATF-2) activation. Because JNKs and p38 MAPKs are generally activated by the same stress signals, they have been referred to as the "stress kinases," and the role of these kinases in regulation of cytokine release and apoptosis are implicated.

Cerebral ischemia induces the increase in intracellular Ca concentration via the activation of glutamate receptors, and the robust activation of a large number of transcription factors and immediate-early genes and late-response genes occurs in neuronal and non-neuronal cells *(5–7).* Although the molecular mechanisms of neuronal injury and recovery after cerebral ischemia have been intensively investigated using various ischemic animal models (particularly, rodent models), the key molecules that determine the fate of neurons remain to be determined, and any therapeutic strategy based on molecular mechanisms have not been proven to be effective in clinical situation by now. In the central nervous system, MAPKs are relatively highly expressed *(8–10),* and several recent papers have reported that the expression or phosphorylation levels of MAPKs drastically change in postischemic brain tissues, and that the inhibition of MAPK cascades can alter the outcome of ischemic brain injury in animal models. The regulation or modulation of gene expression through MAPK cascades may be a novel avenue for the prevention of ischemic neuronal damage and the induction of neuronal functional recovery.

In the current paper, the authors have extensively reviewed the literature, in order to elucidate the role of MAPK cascade in neuronal survival and death in vitro, and in neuronal injury in cerebral ischemia in vivo.

MAPK and Neuronal Death and Survival In Vitro

Many reports have demonstrated that apoptosis significantly contributes to neuronal death and brain tissue damage after cerebral ischemia, although the precise molecular mechanisms that regulate neuronal survival and death have not been well defined in ischemic brain *(11).* The fact that the inhibition of transcription or protein synthesis can prevent neuronal death induced by NGF withdrawal, suggests that *de novo* gene expression may be required for the process to neuronal death *(12).* Caspases comprise a family of cysteine proteases and cytochrome c release from mitochondria, and, following activation of caspase cascades, produce apoptosis in neuronal cells. MAPK superfamily seems to regulate the expression and activity of genes that are involved in the apoptotic pathway and play important roles in neuronal apoptosis. Although the involvement of MAPK cascades in apoptosis and antiapoptosis is implicated in various cells, including neurons, the relationship or crosstalk between caspase cascades and MAPK cascades has not yet been fully elucidated (*see* Table 1).

The JNK/p38 MAPKs pathways and the ERK pathway seem to have opposing effects on neuronal apoptosis in vitro. In pheochromocytoma 12 (PC12) cells, withdrawal of NGF causes apoptosis via the activation of p38 MAPK pathway *(13).* Xia et al. *(14)* showed that NGF withdrawal led to sustained activation of the JNK and p38 enzymes, and sustained inhibition of ERKs in PC12 cells, and also showed that the activation of JNK and p38, and concurrent inhibition of ERK, were critical for induction of apoptosis in these cells, by dominantly interfering with constitutively activated forms of various components in the JNK/p38 and ERK signaling pathways. In their experiments, apoptosis of PC12 cells was reduced by inhibiting p38 MAPK activity. They suggested that a balance between ERK and JNK/p38 might determine cell survival or death, under a given condition.

Deshmukh et al. *(15)* showed that a broad-spectrum caspase inhibitor, bocaspartyl-(OMe)-fluoromethylketone, which protects sympathetic neurons against apoptosis after NGF withdrawal, did not prevent the induction of *c-jun* mRNA. Park et al. *(16)* demonstrated, in neuronal apoptosis by NGF

Table 1
MAPKs and Neuronal Death and Survival In Vitro

Author/year	Type of neurons	Upstream	MAPKs	Downstream	Insult
Neuroprotective	PC12		ERK↓		Apoptosis by NGF withdrawal
Yan/1998 *(36)*	PC12	Ras ↑	ERK ↑		Survival by NAC after serum deprivation
Anderson/1999 *(35)*	Sympathetic neuron		ERK ↑		Neuroprotection from araC induced apoptosis
Mazzoni/1999 *(37)*		Ras ↑	ERK ↑	c-Jun ↓, BAX ↓ p53 ↓	Survival by Ras after NGF withdrawal
Hetman/1999 *(38)*	Cortical neuron		ERK ↑		Survival by BDNF from camptothecin-induced apoptosis
Owada/1999 *(34)*	Cortical neuron		ERK ↑	caspase 3 ↓	Survival by midkine after serum deprivation
Schmid/1999 *(40)*	Cerebellar neuron		ERK ↑		NCAM-induced outgrowth
Bonni/1999 *(39)*	Cerebellar neuron		ERK ↑	CREB ↑, BAD ↓	BDNF-induced survival
Neurotoxic					
Virdee/1995 *(41)*	Sympathetic neuron		ERK ~		Survival by NGF, CNTF, LIF
Park/1999 *(43)*	Cortical neuron		ERK ↑	Egr-1 ↑	Zinc-induced death
Satoh/2000 *(45)*	Cortical neuron hippocampal cell line		ERK ↑		Oxidative stress
Stanciu/2000 *(44)*	Cortical neuron hippocampal cell line		ERK ↑		Glutamate-induced apoptosis
Runden/1998 *(42)*	Hippocampal neuron	MEK1/2 ↑	ERK ↑		Okadaic acid injury
Mukherjee/1999 *(27)*	Hippocampal neuron	PAF ↑	ERK ↑		NMDA and kainate receptor activation
Xia/1995 *(14)*	PC12		JNK ↑		Apoptosis by NGF withdrawal
Kanamoto/2000 *(31)*	PC12	ASK ↑	JNK ↑	c-Jun ↑	Apoptosis by NGF withdrawal
Bazenet/1998 *(17)*	Sympathetic neuron	Cdc42 ↑	JNK ↑	c-Jun ↑	Apoptosis by NGF withdrawal
Eilers/1998 *(19)*	Sympathetic neuron	MEKK1 ↑, SEK1 ~	JNK ↑	c-Jun ↑	Apoptosis by NGF withdrawal
Kanamoto/2000 *(31)*	Sympathetic neuron	ASK ↑	JNK ↑	c-Jun ↑	Apoptosis by NGF withdrawal
Chihab/1998 *(22)*	Fetal forebrain neuron		JNK ↑		Transient hypoxia
Luo/1998 *(18)*	Neonatal striatal neuron 293 cell line	SEK1 ↑	JNK ↑	c-Jun ↑	Dopamine-induced apoptosis
Kawasaki/1997 *(26)*	Cerebellar granule cell		JNK ↑		Glutamate-induced apoptosis
Shimoke/1999 *(25)*	Cerebellar granule cell		JNK ↑	c-Jun ↑	Apoptosis by inhibition of PI3-K activity
Mukherjee/1999	Hippocampal neuron	PAF ↑	JNK ↑		NMDA and kainate receptor activation
Maroney/1998 *(23)*	Motoneuron		JNK ↓		Survival by CEP-1347
Xia/1995 *(14)*	PC12		p38 ↑		Apoptosis by NGF withdrawal
Kummer/1997 *(13)*	PC12		p38 ↑		Apoptosis by NGF withdrawal
Kawasaki/1997 *(26)*	Cerebellar granule cell		p38 ↑		Glutamate-induced apoptosis
Mukherjee/1999 *(27)*	Hippocampal neuron	PAF ↑	p38 ↑		NMDA and kainate receptor activation
Heidenreich/1996 *(30)*	Fetal forebrain neuron		p38 ↓		Survival by insulin
Ghatan/2000 *(28)*	Cortical neuron neuroblastoma cell line		p38 ↑	Bax ↑	No donor-induced apoptosis

NGF, nerve growth factor; NAC, N-acetylcysteine; araC, cytosine arabinoside; BDNF, brain-derived neurotrophic factor; NCAM, neural cell adhesion molecule; CREB, cAMP response element-binding protein; CNTF, ciliary neurotrophic factor; LIF, leukemia inhibitory factor; PAF, platelet activating factor.

withdrawal of PC12 cells, that bcl-2 and a number of survival-promoting agents, such as aurintricarboxylic acid, N-acetylcysteine, the nitric oxide (NO) generator diethylenetriamine NO, 8-bromo-cyclic guanosine monophosphate, and 8-(4-chlorophenylthio)-cAMP, prevented apoptosis with inhibition of JNK activation, and that zVAD-fluoromethylketone (a permeant ICE family inhibitor), actinomycin D, and the G1/S cell cycle inhibitor, deferoxamine, prevented apoptosis without JNK inhibition. These data suggest that the induction in PC12 cells of *c-Jun* mRNA, after NGF withdrawal, does not necessarily require caspase activation, and that a signal pathway that regulates c-Jun expression may be located in an upstream or independent pathway of caspase activation in neuronal cells.

Although there is controversy whether the JNK pathway is a requisite in the process of neuronal apoptosis induced by caspase activation, several reports suggest that the activation of JNK pathway is involved in neuronal apoptosis induced by several insults. Bazenet et al. *(17)* demonstrated that apoptosis, by NGF withdrawal of rat sympathetic neuron, required the activity of the small guanosine triphosphate-binding protein, Cdc42, and the overexpression of an active form of Cdc42 was sufficient to mediate neuronal apoptosis via the activation of c-Jun pathway. Luo et al. *(18)* showed, by using 293 cell line and primary neonatal rat postmitotic striatal neuron cultures, that dopamine induced apoptosis in a time- and concentration-dependent manner, and that, concomitant with apoptosis, dopamine induced the activation of JNK pathway, including increases in JNK activity, phosphorylation of c-Jun, and subsequent increases in c-Jun. They also showed that transient expression of a dominant-negative mutant, SEK1, an upstream kinase of JNK, and of a dominant-negative c-Jun mutant, prevented dopamine-induced apoptotic cell death. Eilers et al. *(19)* also showed in sympathetic neurons that activation of Jun kinase and increases in c-Jun phosphorylation and c-Jun levels occurred at the same time after NGF withdrawal, and that c-Jun levels and phosphorylation were regulated by an SEK1-independent pathway.

Although JNK inhibitors are not widely available, Maroney et al. *(20)* demonstrated that a novel selective inhibitor of the JNK signaling pathway, CEP-1347, rescued sympathetic neurons and neuronally differentiated PC12 cells from death induced by NGF withdrawal, exposure to ultraviolet irradiation, and subjection to oxidative stress. Moreover, Behrens et al. *(21)* demonstrated that mutant Jun-expressing mice were resistant to neuronal apoptosis induced by the excitatory amino acid, kainate, and that primary, mutant Jun-expressing neurons were also protected from apoptosis and exhibited unaltered JNK activity. They suggested that c-Jun was the essential substrate of JNK signaling during kainate-induced neuronal apoptosis. JNK pathway seems to be involved not only in mature neurons, but also in neurons from developing brains. Chihab et al. *(22)* showed, in cultured neurons isolated from the fetal rat forebrain, that transient hypoxia of 6 h might trigger apoptosis, through the activation of JNK signaling pathway, and Maroney et al. *(23)* demonstrated, in cultured embryonic motoneurons, that CEP-1347 blocked apoptosis induced by withdrawal of trophic factors.

The phosphatidylinositol 3-kinase (PI3K)-Akt pathway has been reported to inhibit apoptosis and promote cell survival, through the inactivation of Bad and caspase-9 *(24).* Shimoke et al. *(25)* demonstrated that apoptosis was induced by inhibition of PI3K activity with LY294002, an inhibitor of PI3K in cerebellar granule neurons, and that PI3K inhibition induced the suppression of Akt activity and elevation of the JNK activity in these cells, and suggested that the PI3K-Akt pathway prevented neuronal cell death by suppressing the activation of JNK and c-Jun expression.

The overstimulation of glutamate receptors are supposed to be involved in ischemic neuronal cell death. Recent reports have suggested that the p38 pathway is activated through glutamate receptors in neuronal cells. Kawasaki et al. *(26)* showed, in cerebellar granule cells, that

glutamate-induced apoptosis activates the p38 pathway, through the activation of N-methyl-D-aspartate (NMDA) receptor and Ca influx, and that SB203580, a specific inhibitor for p38, inhibited glutamate-induced apoptosis. They concluded that p38 might be involved in glutamate-induced apoptosis in cerebellar granule cells. Mukherjee et al. *(27)* showed that the stimulation of NMDA receptor and kainate receptor activated JNK, p38, and ERK via platelet-activating factor, and brought cell death in primary cultures of hippocampal (HC) neurons.

The activation of p38 pathway seems to induce caspase activation. Ghatan et al. *(28)* reported, using the model of NO-mediated cell death in human neuroblastoma cells and in primary cultures of cortical neurons, that a p38 inhibitor, SB203580, blocked intracellular Bax translocation, diminished caspase induction, and significantly enhanced neuronal survival, and suggested that p38 activity might play a critical role in NO-mediated neuronal cell death, by stimulating Bax translocation to the mitochondria, thereby activating the cell death pathway.

There are some specific inhibitors for p38 pathway, and Horstmann et al. *(29)* tested the effects of two selective p38 inhibitors, the pyridinyl imidazole compounds, SB203580 and SB202190, on the in vitro survival of sensory, sympathetic, ciliary, and motor embryonic neurons, after withdrawal of growth factors, and showed that these inhibitors promoted cell survival in a dose-dependent fashion. Heidenreich and Kummer *(30)* showed that insulin promoted the survival of cultured forebrain neurons, concomitant with inhibition of p38, in the absence of the effects on ERK and JNK activity. Kummer et al. *(13)* also demonstrated in PC12 cells that neuronal cell death, by NGF withdrawal accompanied p38 activation, that p38 inhibition blocked cell death, and that insulin promoted cell survival by inhibiting the p38 pathway, and not by stimulating ERK.

Recently, a new MAPK kinase kinase, apoptosis signal-regulating kinase 1 (ASK1), which activates both the JNK and p38 pathways, and plays pivotal roles in tumor necrosis factor (TNF)- and Fas-induced apoptosis, has been identified. Kanamoto et al. *(31)* demonstrated that overexpression of ASK1-DeltaN, a constitutively active mutant of ASK1, activated JNK and induced apoptosis in differentiated PC12 cells and primary rat sympathetic neurons. Moreover, they showed that, in differentiated PC12 cells, NGF withdrawal induced a increase in the activity of endogenous ASK1, and the expression of a kinase-inactive ASK1 significantly blocked both NGF withdrawal- and Cdc42-induced neuronal death and c-jun activation. Since JNK and p38 pathways are activated by ASK1, ASK1 can be a key molecule for controlling neuronal apoptosis.

Although there is a general consensus that JNK and p38 pathways induce neuronal death, there are some reports which showed that the activation of JNK and p38 pathways might be involved in neuronal cell survival. Roulston et al. *(32)* demonstrated that TNF induced biphasic activation of JNK/p38 pathways and multiple effects, including proliferation, differentiation, and apoptosis, and that the inhibition of early TNF-induced JNK and p38 kinases activation, using MKK4/MKK6 mutants or the p38 inhibitor, SB203580, increased TNF-induced apoptosis; expression of wild-type MKK4/MKK6 enhanced survival. They concluded that early activation of p38 kinase, initiated by TNF, may be necessary to protect cells from TNF-mediated cytotoxicity, although late activation of JNK/p38 pathways was coincident with apoptosis, and was caspase-dependent. These data suggest that the roles of MAPK cascades on neuronal death and survival depend on the timing of expression in a given condition.

Several studies have showed that the activation of ERK pathway produces neuroprotective effects *(33).* Recently, Owada et al. *(34)* demonstrated that neuronal apoptosis, induced by serum deprivation, was accompanied with the activation of caspase-3 in primary neuronal cultures isolated from mouse cerebral cortex, and that midkine, a new mem-

ber of the heparin-binding neurotrophic factor family, inhibited the induction of apoptosis and the activation of caspase-3 in a dose-dependent manner, possibly through activating Akt and ERK. Anderson et al. *(35)* demonstrated that the inhibition of ERK pathway by PD98059 resulted in a significant increase in the rate of apoptosis induced by antimitotic nucleoside, cytosine arabinoside, in rat sympathetic neurons, without affecting NGF-maintained signaling. Yan and Greene *(36)* showed that N-acetylcysteine (NAC) promoted survival of PC12 cells, in the absence of trophic factors, and that the promotion of PC12 cell survival by NAC was totally blocked by PD98059, an inhibitor of ERK, suggesting a required role for ERK activation in the NAC mechanism. They also showed that NAC activated ERK pathway in these cells, and survival induced by NAC was not promoted in serum-deprived PC12 cells constitutively expressing a dominant-negative form of Ras, which is located upstream of ERK.

Mazzoni et al. *(37)* demonstrated, in sympathetic neurons, that a Ras effector mutant, which selectively stimulates PI3K and Akt, rescued neuronal survival in the absence of NGF by suppressed c-jun, BAX, and p53 levels, and that the PI3-K inhibitor, LY 294002, inhibited both Ras- and NGF-dependent survival. They also showed that another Ras mutant, which activated MEK/MAPK, but not PI3K/Akt, was less effective in rescuing survival, but the MEK inhibitor, PD98059, also partially suppressed Ras-dependent survival, suggesting that Ras-induced neuroprotective effect was mediated by activation of both the PI3K- and partially ERK-signaling cascades. Hetman et al. *(38)* showed that BDNF protected cortical neurons against apoptosis induced by campothecin or serum deprivation, and activated the ERK and the PI3K pathways, and that the inhibition of ERK pathways increased neuronal apoptosis by camptothecin, and the inhibition of PI3K pathway increased apoptosis by serum deprivation. Bonni et al. *(39)* reported that BDNF activated ribosomal S6 kinase (RSK) via ERK pathway in BDNF-induced survival of cultured rat cerebellar granule neurons. They demonstrated that the MAPK-activated kinases, the RSKs, catalyzed the phosphorylation of the proapoptotic protein, Bad, at Ser 112, and that the Rsk-induced phosphorylation of Bad at Ser 112 suppressed Bad-mediated apoptosis in neurons, and that RSKs phosphorylated the transcription factor, CREB, at Ser 133 and activated CREB-promoted cell survival. They suggested that ERK pathway promoted cell survival by a dual mechanism comprising the posttranslational modification and inactivation of a component of the cell death machinery and the increased transcription of prosurvival genes.

These results suggest that the ERK pathway is one of several neuroprotective mechanisms that are activated by stress to counteract death signals in central nervous system neurons. The relative contribution of the ERK and PI3K-Akt pathways to neuronal survival may depend on cell types, insults, and conditions. Schmid et al. *(40)* demonstrated, in rat cerebellar neurons, that clustering of neural cell adhesion molecule (NCAM) in neural plasma membrane stimulated the phosphorylation of ERK1/2 through the activation of Ras, MAPK kinase, and further activated CREB, and that inhibition of MAPK cascade reduced NCAM-dependent neurite outgrowth.

On the other hand, there are some reports which show that ERK pathway does not have neuroprotective effects, but even has neurotoxic effects. Virdee and Tolkovsky *(41)* reported that ERK activation was neither necessary nor sufficient for cell survival induced by NGF, ciliary neurotrophic factor, and leukemia inhibitory factor and the cAMP analog, and that alternative pathways existed for effecting long-term survival of rat sympathetic neurons. Runden et al. *(42)* demonstrated, in HC slice cultures, that okadaic acid, which caused a dose- and time-dependent selective injury to HC pyramidal cells in the CA3 region and granule cells in the dentate gyrus, led to a rapid and sustained phosphorylation of the ERK1/2, and the MAPK kinase (MEK1/2)

inhibitor, PD98059, ameliorated okadaic acid-induced cell death.

These results indicate that sustained activation of ERK pathway may be selectively harmful in specific subsets of neurons. Park and Koh *(43)* reported, in mouse cortical cultures, that zinc induced neuronal death concomitant with Egr-1 expression and ERK activation, and that PD98059 blocked ERK activation, Egr-1 induction, and neuronal death by zinc. More recently, Stanciu et al. *(44)* reported that the delayed and persistent activation of ERK was associated with glutamate-induced oxidative toxicity in a neuronal cell line and primary cortical neuron, and Satoh et al. *(45)* reported, in a mouse HC cell line (HT22) and rat primary cortical cultures, that a MAPK/ERK kinase-specific inhibitor, U0126, protected HT22 cells against oxidative stress induced by glutamate, and also protected rat primary cultured cortical neurons against glutamate or hypoxia.

Table 1 summarizes the relationship between MAPK and neuronal death and survival in vitro. These data suggest that ERK pathway has both protective and toxic effects, and JNK and p38 pathways have mainly toxic effect on neuron. The opposing effects of ERK pathway may result from types of cells or kinds of insults. ERK pathway seems to be a key molecule in growth-factor-dependent neuronal survival, but neurotoxicity induced by glutamate, oxidative stress also seems to use ERK pathway, in a given condition. Downstream cascades after the activation of MAPK pathway, and crosstalks with other pathways, such as PI3K-Akt, should be determined, to clarify the precise roles of MAPK cascade.

MAPK and Global Ischemia In Vivo

A brief period of global cerebral ischemia causes neuronal cell death in the region vulnerable to ischemia, such as CA1 pyramidal cell layer of the HC, several days after ischemia. The HC neurons seem to be normal during the first 24 h after transient ischemia. This has been called "delayed neuronal cell death" (DND) *(46),* which has been suggested to be apoptotic, based on the evidence of DNA fragmentation, fragmentation of the nucleus, cell shrinkage, and chromatin condensation *(47–49).* The mechanism of DND has not been fully elucidated, but there are some evidences that DND is associated with the activation of MAPK cascades (*see* Table 2).

Several in vivo studies demonstrated that JNK/p38 pathways were activated in the CA1 region after global ischemia, and the inhibition of JNK/p38 pathways might rescue CA1 neurons from DND. Walton et al. *(50)* reported the effects of cerebral ischemia on MAPK cascades, and demonstrated, in gerbil HC after 7 min global ischemia, that activated p38 MAPK activity and MAPKAP2, downstream of p38 pathway, activity, increased over 4 d after ischemia. They showed that activated p38 MAPK immunoreactivity was observed in microglia in regions adjacent to, but not in, the dying CA1 neurons, and neither JNK 1 nor ERK activity was altered after ischemia. Gillardon et al. *(51)* showed that nuclear phospho-c-Jun immunoreactivity became apparent selectively in vulnerable HC CA1 neurons at 24 h after transient global cerebral ischemia. Recently, the authors demonstrated, in gerbil HC after 5 min forebrain ischemia, that JNK and p38 were activated at 15 min of reperfusion, then gradually reduced and disappeared in neurons of CA1 region (Fig. 2), and pretreatment with SB203580, a p38 inhibitor, reduced ischemic cell death in the CA1 neurons, by inhibiting the activity of p38 (*52;* Fig. 3), and also showed, in a mouse transient forebrain ischemia model, that JNK/p38 pathway were activated in the HC *(53).* Yang et al. *(54)* demonstrated, in an in vivo seizure model, that the disruption of the gene encoding JNK3 in mice caused the mice to be resistant to the excitotoxic glutamate-receptor agonist kainic acid: They showed the reduction in seizure activity and HC neuron apoptosis by kainic acid, suggesting that knockout of the JNK3 locus prevents excitotoxic cell death of HC neurons.

Table 2
MAP Kinases and Global Ischemia In Vivo

Author/year	Animal	Upstream	MAPKs	Downstream	Localization	Temporal distribution
Hu/1994 *(56)*	Rat		ERK↑		CA3, DG	
Shioda/1998 *(58)*	Rat	IL-6	JNK↓,ERK↑		CA1	During neuroprotection by PACAP
Gillardon/1999 *(51)*	Rat		JNK↑	PAG608↑,c-Jun↑	CA1	24 h after ischemia
Ozawa/1999 *(55)*	Rat		JNK↑,p38↑,ERK↑		CA1	
Hu/2000 *(57)*	Rat	Trk↑	ERK↑		DG	30 min and 4 h after reperfusion
		Trk slightly↑	ERK.~		CA1	30 min and 4 h after reperfusion
				ATF-2↑, c-Jun↑	CA1	Late period of reperfusion
Walton/1998 *(50)*	Gerbil		p38↑	MAPKAP2↑	CA1(microglia)	Up to 4 d after ischemia
Sugino/2000 *(52)*	Gerbil		JNK↑,p38↑		CA1	15 min after reperfusion
			JNK↑,p38↑		CA3, DG	Peaked at 6h after referfusion
			ERK↑		CA3, DG	Transiently
Tsuji/2000 *(59)*	Gerbil		JNK↑,p38↑,ERK↑		CA1	During neuroprotection by PBN

DG, dentate gyrus; PACAP:pituitary adenylate cyclase-activating polypeptide; PBN:alpha-Phenyl-N-tert-butylnitrone; PAG608:p53-activated gene 608; 2L-6:interleukin 6; Trk:thyrosine kinase; MAPKAP2:MAPK-activated protein 2; AFT-2:activating transcription factor 2.

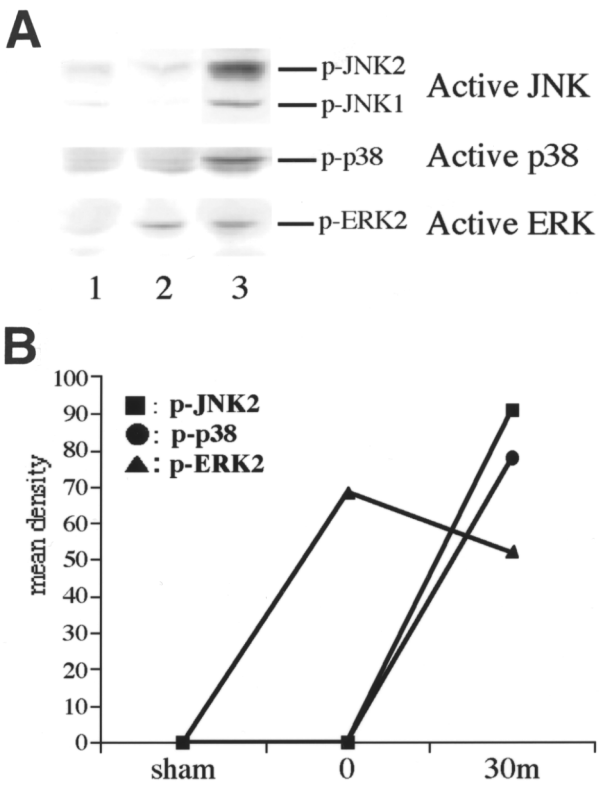

Fig. 2. Increased phosphorylation of JNK, p38, and ERK after 5 min of transient forebrain ischemia. Western immunoblots of HC homogenates of sham-operated gerbils (lane 1) and postischemic gerbils at 0 min (lane 2) and 30 min (lane 3), after 5 min of transient forebrain ischemia (**A,** upper panel: active JNK, middle panel: active p38, lower panel: active ERK. p-, phosphorylated). Quantitative representation of immunoblots, showing activations of JNK1 and p38 30 min after ischemia, and ERK2 immediately after ischemia in the HC extracts **(B).**

Ozawa et al. *(55)* reported that ischemia-reperfusion led to a strong activation of the JNK, ERK, and p38 enzymes in the CA1 region of the rat HC, after transient global ischemia produced by 5 min of cardiac arrest.

As for ERK pathway, ERK seems to be activated, not in CA1 region, but in CA3 and dentate gyrus. Hu and Wieloch *(57)* demonstrated, in rat transient forebrain ischemia, that ERK was markedly phosphorylated and activated immediately following ischemia, particularly in the CA3 region and dentate gyrus, but not in the striatum, and the phosphorylation of ERK was less intense, and decreased later, during reperfusion in the CA1 region. The authors demonstrated, in gerbil transient ischemia, that ERK was activated transiently in CA3 fibers and dentate gyrus, and that an ERK inhibitor PD98059 did not reduce ischemic cell death in the CA1 region (*53;* Figs. 2 and 3). Hu et al.*(58)* reported, in rat 15-min transient forebrain ischemia, that tyrosine phosphorylation of Trk kinase, an ERK upstream growth factor receptor, was elevated in the dentate gyrus and, to a lesser extent, in the CA1 region, and that the phosphorylation of ATF-2 and c-Jun was selectively increased in CA1 dying neurons during the late period of reperfusion.

These reports suggest that the ERK pathway might have neuroprotective effects on the dentate granule cells and CA3 region, and that the activation of JNK/p38 pathways in CA1 dying neurons may bring damage signals in these neurons and that the lack of protective signals, acting in company with the presence of damage signals in CA1 neurons after ischemia, may contribute to DND after transient forebrain ischemia.

Some protective agents against DND are reported to act by inhibiting JNK/p38pathway. Shioda et al. *(59)* demonstrated that either intracerebroventricular or intravenous infusion of pituitary adenylate cyclase-activating polypeptide (PACAP) prevented the ischemia-induced apoptosis of neurons in the CA1 region of rat HC, and that PACAP inhibited the activation of JNK after ischemic stress by secretion of interleukin-6 into the cerebrospinal. Tsuji et al. *(59)* showed in gerbil HC that intraperitoneal injection of α-phenyl-N-tert-butylnitrone, which protects HC neurons against DND, enhanced the activation of ERK, and suppressed the activation of JNK and p38, with the activation of HSP27 and HSP70 at 6 h after ischemia. These results support the hypothesis that JNK/p38 pathways play important roles on DND after transient global ischemia in vivo, although which pathway plays more important roles on DND has not

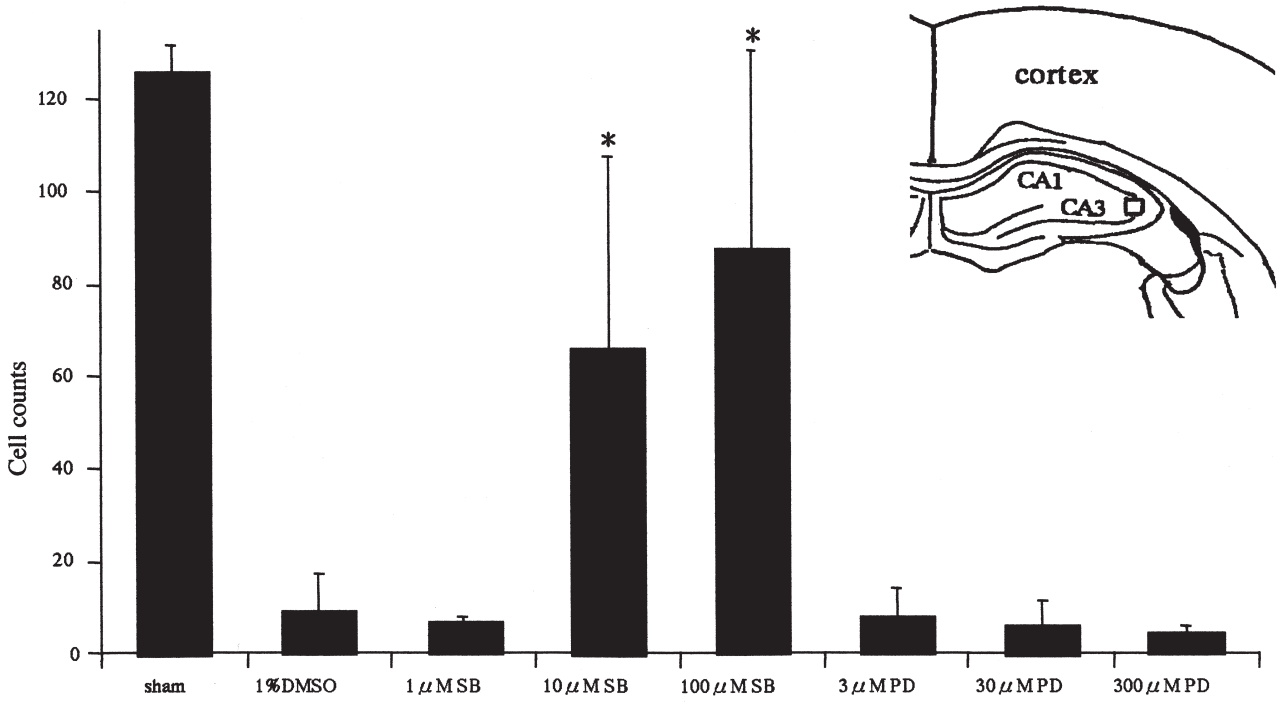

Fig. 3. The effects of SB203580 and PD98059 on neuronal death in CA1 region. Numbers of survival neurons in CA1 region of the HC, after SB203580 and PD98059 treatments at various doses. SB203580 showed neuroprotective effects in a dose-dependent manner, in CA1 subfield. Neuroprotective effects with 10 μ*M* and 100 μ*M* SB203580 were significant, compared with vehicle (1% dimethyl sulfoxide) (*$p < 0.0001$). PD98059 showed no neuroprotective effects in CA1 region.

been fully understood, because of lack of specific inhibitors for JNK.

MAPKs and Focal Ischemia In Vivo

Middle cerebral artery occlusion (MCAO) leads to localized brain infarction in cerebral hemisphere and basal ganglia. Recently, many reports have shown that cells within the ischemic regions die, not only by necrosis, but also by apoptosis *(60–62).* Li et al. *(63)* demonstrated, in transient MCAO induced by means of an intraluminal monofilament, that the ratio of apoptotic to necrotic cells was 1:9, 1:6, 1:13 in the ischemic core, and inner and outer boundary zones, respectively at 46 h, after 2 h of MCAO. The presence of apoptotic neuronal death after focal brain ischemia implies that MAPK cascades may activate in the focal ischemic region in brain (*see* Table 3).

Herdegen et al. *(64)* reported that prolonged N-terminal phosphorylation of c-Jun was detected in affected neurons up to 5 d after transient focal ischemia, and that the phosphorylation of c-Jun was linked with the expression of Fas-ligand (APO-1, CD95-ligand), whose gene is a putative c-Jun/AP-1 target, and with TUNEL reactivity, a marker for apoptosis. Martin-Villalba et al. *(65)* reported that ATF-2, a main target molecule for JNK, was suppressed in neurons within 1–4 h following transient focal ischemia by MCAO, and regained basal levels between 12 and 72 h following ischemia, suggesting that the rapid and persistent downregulation of ATF-2 is a

Table 3
MAP Kinases and Focal Ischemia In Vivo

Author/year	Animal	Upstream	MAPKs	Downstream	Localization	Temporal distribution
Herdegen/1998 *(64)*	Rat		JNK↑	c-Jun↑,Fas ligand↑	Ischemic region	Up to 5 d after ischemia
Martin/1998 *(65)*	Rat			ATF-2↓	Ischemic region	1–4 h after ischemia
Kitagawa/1999 *(66)*	Rat		ERK↑		Ischemia region	Activated at 3 and 8 h and decreased at 24 h
			ERK↑		Penumbral region	Activated at 3 and 8 h and continuously expressed at 24 h
Alessandrini/1999 *(70)*	Mouse	MEK1_	ERK↑			
Irving/2000 *(67)*	Rat		ERK↑	CREB↑	Penumbral region	Up to 24 h
			p38↑		Core and penumbral region (astroglia)	Up to 24 h
Wu/2000 *(68)*	Mouse		JNK↑,p38↑,ERK↑		Ischemic neuron and astroglia	Up to several h after ischemia

ATF-2:activating transcription factor 2; CREB:cyclic AMP/responsive element-binding protein calcium.

Table 4
MAP Kinases and Ischemic Tolerance

Author/year	Model	Upstream	MAPKs	Downstream	Temporal distribution
Tauskela/1999 *(89)*	Neuron culture		ERK.~		
Gonzalez/2000 *(91)*	Neuron culture	Ras↑	ERK↑		
Shamloo/1999 *(90)*	Rat CA1	MEK↑	ERK↑		Elevated at 30 min, 4h, 2d after sublethal ischemia
Gu/2000 *(92)*	Rat CA1		ERK↑		After sublethal ischemia
Sugino/2000 *(88)*	Gerbil CA1		JNK↑		After 3-NP administration

3-NP: 3-nitropropionic acid.

constituent of the long-term neuronal stress response, and that the reappearance of ATF-2, after weeks, is a marker for the normalization of neuronal gene transcription following brain injury. Kitagawa et al. *(66)* showed, in rat focal ischemia, that robust expression of ERK was noted at 3 and 8 h, in most neurons in the area of ischemia, and that ERK continued to be expressed in the ischemic penumbra, but decreased in the ischemic core at 24 h after ischemia, and suggested the neuroprotective role of ERK against focal cerebral ischemia. Irving et al. *(67)* reported, in rat transient and permanent MCAO models, that phospho-p38 immunostaining was markedly increased in cells with astrocyte-like morphology in both core and penumbral-like regions, and phospho-ERK1/2 staining was markedly increased within the cytoplasm of neuronal perikarya in penumbral-like regions. Recently, Wu et al. *(68)* demonstrated, in a mouse permanent MCAO model, that the transient and distinct activation of JNK, p38, and ERK were observed in neurons and astroglial cells in ischemic regions.

The inhibition of MAPK cascades may be a potential therapeutic strategy for focal cerebral ischemia. As for JNK/p38 pathways, Barone and Feuerstein *(69)* demonstrated that SB239063, a p38 inhibitor, significantly reduced infarct volume, and improved neurological functions in a rat focal MCAO model. On the other hand, ERK activation, which is generally attributed to neuronal cell survival and proliferation, might be neurotoxic in focal cerebral ischemia. Alessandrini et al. *(70)* demonstrated that treatment of mice, 30 min before ischemia, with a MEK1-specific inhibitor, PD98059, dramatically reduced focal infarct volumes at 22 h after transient MCAO, suggesting a important role of ERK pathway in the evolution of cerebral ischemia.

MAPKs and Ischemic Tolerance

Preceding sublethal ischemia induces ischemic tolerance to subsequent lethal ischemic stress in HC CA1 pyramidal neurons *(71,72).* The establishment of ischemic tolerance seems to require the cellular signal transduction and gene expression. The molecular basis of tolerance induction is not yet determined, but a role for stress proteins *(73,74),* bcl-2 oncoprotein *(75),* Jun-related protein *(76),* opening of K^+ channels *(77),* and adenosine receptor activation *(78)* have been suggested. Recent papers imply that MAPK cascades have also been involved in producing ischemic tolerance in the brain (*see* Table 4).

Ischemic tolerance was first described in the heart *(79),* and later in rat and gerbil brains. In the heart, it has been reported that the phosphorylation of Tyr 182 of p38 correlates with the protective action of preconditioning in the heart *(80–82).* Baines et al. *(83)* suggested that p38 and/or JNK played important roles in ischemic preconditioning in the rabbit heart,

using anisomycin, a p38/JNK activator in vivo and in vitro. Haq et al. *(84)* demonstrated that activation of p38 was involved in adenosine-mediated ischemic preconditioning in the perfused rat heart. These observations indicate that JNK and p38 play important roles in ischemic preconditioning in the heart.

In the brain, Sommer et al. *(85)* demonstrated, in CA1 neurons of gerbil HC, that sublethal ischemia caused a postischemic expression of Jun, but not Fos, protein. Kato et al. *(86)* demonstrated that prominent Jun immunoreactivity was induced in astrocytes in the CA1 region of the gerbils with ischemic tolerance, and suggested that Jun might contribute to the ischemic tolerance by inducing changes in gene expression in astrocytes. Recently, the authors demonstrated, in CA1 region of gerbil HC, that intraperitoneal administration of 3-nitropropionic acid, a mitochondria toxin, induced JNK activation and a tolerant effect to subsequent lethal ischemia, and prevented DND *(87).* These data support the possible involvement of JNK pathway in ischemic tolerance in the brain.

Although Tauskela et al. *(88)* showed that ischemic preconditioning of neurons did not require activation of PKC and ERK in rat cortical cultures, some reports have shown that the ERK pathway may be related with ischemic tolerance in vivo and vitro. Shamloo et al. *(89)* demonstrated that increased phosphorylation of both MEK and ERK were found in neuronal cell bodies, particularly in the nucleus, after preconditioning ischemia in the rat HC CA1 region, and, during the early reperfusion period (30 min), after the second ischemic insult, the phosphorylation levels of these two kinases were increased in both nonconditioned and preconditioned brains. They concluded that the phosphorylation of the ERK cascade after sublethal ischemia correlated with the neuroprotection induced by preconditioning. Gonzalez-Zulueta et al. *(90)* reported using cortical neuron cultures that oxygen-glucose deprivation (OGD) induced Ras activation in an NMDA receptor- and NO-dependent manner, and that Ras activity was necessary and sufficient for OGD tolerance in neurons and downstream of Ras-ERK cascade was required for OGD preconditioning. Recently, Gu et al. *(91)* demonstrated that ERK, but not JNK, was activated after preconditioning ischemia, and the increased JNK1, but not ERK, diphosphorylation, after lethal ischemia, was eliminated by pretreatment with preconditioning ischemia in rat. They suggested that the elimination of JNK1 activation after lethal ischemia, by preconditioning ischemia, may be one of the important protective mechanisms in ischemic tolerance, and ERK activation may be involved in the induction of the protective responses.

Conclusions

Although MAPK cascades have been intensively studied in neuronal apoptosis and cerebral ischemia, target molecules of MAPK cascades are not yet defined, and the activity of MAPK cascade is regulated by other signal transduction pathways, and the roles of each cascade on neuronal death and survival and ischemic tolerance seem to be altered by the experimental designs and models used. Further studies are necessary before MAPK cascades can be a potential target pathway for the regulation of neuronal fate after cerebral ischemia.

References

1. Davis R. J. (1993) The mitogen-activated protein kinase signal transduction pathway. *J. Biol. Chem.* **268,** 14,553–14,556.
2. Nishida E. and Gotoh Y. (1993) The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem. Sci.* **18,** 128–131.
3. Seger R. and Krebs E. G. (1995) The MAPK signaling cascade. *FASEB J.* **9,** 726–735.
4. Hunter T. (1995) Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* **80,** 225–236.
5. Dirnagl U., Iadecola C., and Moskowitz M. A. (1999) Pathobiology of ischemic stroke: an integrated view. *Trends Neurosci.* **22,** 391–397.

6. Nowak T. S., Jr. and Kiessling M. (1999) Reprogramming of gene expression after ischemia, in *Cerebral Ischemia: Molecular and Cellular Pathophysiology* (Walz W., ed.), Humana, Totowa, NJ, pp. 145–216.
7. Sharp F. R., Lu A., Tang Y., and Millhorn D. E. (2000) Multiple molecular penumbras after focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* **20,** 1011–1032.
8. Fiore R. S., Bayer V. E., Pelech S. L., Posada J., Cooper J. A., and Baraban J. M. (1993) p42 mitogen-activated protein kinase in brain: prominent localization in neuronal cell bodies and dendrites. *Neuroscience* **55,** 463–472.
9. Thomas K. L. and Hunt S. P. (1993) The regional distribution of extracellularly regulated kinase-1 and -2 messenger RNA in the adult rat central nervous system. *Neuroscience* **56,** 741–757.
10. Ortiz J., Harris H. W., Guitart X., Terwillinger R. Z., Haycock J. W., and Nestler E. J. (1994) Extracellular signal-regulated protein kinases (ERKs) and ERK kinase (MEK) in brain: regional distribution and regulation by chronic morphine. *J. Neurosci.* **15,** 1285–1297.
11. Roy M. and Sapolsky R. (1999) Neuronal apoptosis in acute necrotic insults: why is this subject such a mess? *Trends Neurosci.* **11,** 419–422.
12. Martin D. P., Schmidt R. E., DiStefano P. S., Lowry O. H., Carter J. G., and Johnson E. M., Jr. (1998) Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. *J. Cell Biol.* **106,** 829–844.
13. Kummer J. L., Rao P. K., and Heidenreich K. A. (1997) Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. *J. Biol. Chem.* **272,** 20,490–20,494.
14. Xia Z., Dickens M., Raingeaud J., Davis R. J., and Greenberg M. E. (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* **270,** 1326–1331.
15. Deshmukh M., Vasilakos J., Deckwerth T. L., Lampe P. A., Shivers B. D., and Johnson E. M., Jr. (1996) Genetic and metabolic status of NGF-deprived sympathetic neurons saved by an inhibitor of ICE family proteases. *J. Cell. Biol.* **135,** 1341–1354.
16. Park D. S., Stefanis L., Yan C. Y. I., Farinelli S. E., and Greene L. A. (1996) Ordering the cell death pathway. Differential effects of BCL2, an interleukin-1-converting enzyme family protease inhibitor, and other survival agents on JNK activation in serum/nerve growth factor-deprived PC12 cells. *J. Biol. Chem.* **271,** 21,898–21,905.
17. Bazenet C. E., Mota M. A., and Rubin L. L. (1998) The small GTP-binding protein cdc42 is required for nerve growth factor withdrawal-induced neuronal death. *Proc. Natl. Acad. Sci. USA* **95,** 3984–3989.
18. Luo Y., Umegaki H., Wang X., Abe R., and Roth G. S. (1998) Dopamine induces apoptosis through an oxidation-involved SAPK/JNK activation pathway. *J. Biol. Chem.* **273,** 3756–3764.
19. Eilers A., Whitfield J., Babij C., Rubin L. L., and Ham J. (1998) Role of the Jun kinase pathway in the regulation of c-Jun expression and apoptosis in sympathetic neurons. *J. Neurosci.* **18,** 1713–1724.
20. Maroney A. C., Finn J. P., Bozyczko-Coyne D., O'Kane T. M., Neff N. T., Tolkovsky A. M., et al. (1999) CEP-1347(KT7515), an inhibitor of JNK activation, rescues sympathetic neurons and neuronally differentiated PC12 cells from death evoked by three distinct insult. *J. Neurochem.* **73,** 1901–1912.
21. Behrens A., Sibilia M., and Wagner E. F. (1999) Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. *Nat. Gene.* **21,** 326–329.
22. Chihab R., Ferry C., Koziel V., Monin P., and Daval J. L. (1998) Sequential activation of activator protein-1-related transcription factors and JNK protein kinases may contribute to apoptotic death induced by transient hypoxia in developing brain neurons. *Brain Res.* **63,** 105–120.
23. Maroney A. C., Glicksman M. A., Basma A. N., Walton K. M., Knight E., Jr., Murphy C. A., et al. (1998) Motoneuron apoptosis is blocked by CEP-1347 (KT 7515), a novel inhibitor of the JNK signaling pathway. *J. Neurosci.* **18,** 104–111.
24. Del Peso L., Gonzalez-Garcia M., Page C., Herrera R., and Nunez G. (1997) Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. *Science* **278,** 687–689.
25. Shimoke K., Yamagishi S., Yamada M., Ikeuchi T., and Hatanaka H. (1999) Inhibition of phosphatidylinositol 3-kinase activity elevates c-Jun N-terminal kinase activity in apoptosis of cultured cerebellar granule neurons. *Dev. Brain Res.* **112,** 243–253.
26. Kawasaki H., Morooka T., Shimohama S., Kimura J., Hirano T., Gotoh Y., and Nishida E. (1997) Activation and involvement of p38 mitogen-activated protein kinase in glutamate-

induce apoptosis in rat cerebellar granule cells. *J. Biol. Chem.* **272,** 18,518–18,521.
27. Mukherjee P. K., DeCoster M. A., Campbell F. Z., Davis R. J., and Bazan N. G. (1999) Glutamate receptor signaling interplay modulates stress-sensitive mitogen-activated protein kinases and neuronal cell death. *J. Biol. Chem.* **274,** 6493–6498.
28. Ghatan S., Larner S., Kinoshita Y., Hetman M., Patel L., Xia Z., Youle R. J., and Morrison R. S. (2000) p38 MAP kinase mediates bax translocation in nitric oxide-induced apoptosis in neurons. *J. Cell Biol.* **150,** 335–348.
29. Horstmann S., Kahle P. J., and Borasio G. D. (1998) Inhibitors of p38 mitogen-activated protein kinase promote neuronal survival in vitro. *J. Neurosci. Res.* **52,** 483–490.
30. Heidenreich K. A. and Kummer J. L. (1996) Inhibition of p38 mitogen-activated protein kinase by insulin in cultured fetal neurons. *J. Biol. Chem.* **271,** 9891–9894.
31. Kanamoto T., Mota M., Takeda K., Rubin L. L., Miyazono K., Ichijo H., and Bazenet C. E. (2000) Role of apoptosis signal-regulating kinase in regulation of the c-Jun N-terminal kinase pathway and apoptosis in sympathetic neurons. *Mol. Cell Biol.* **20,** 196–204.
32. Roulston A., Reinhard C., Amiri P., and Williams L. T. (1998) Early activation of c-Jun N-terminal kinase and p38 kinase regulate cell survival in response to tumor necrosis factor alpha. *J. Biol. Chem.* **273,** 10,232–10,239.
33. Fukunaga K. and Miyamoto E. (1998) Role of MAP kinase in neurons. *Mol. Neurobiol.* **16,** 79–95.
34. Owada K., Sanjo N., Kobayashi T., Mizusawa H., Muramatsu H., Muramatsu T., and Michikawa M. (1999) Midkine inhibits caspase-dependent apoptosis via the activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase in cultured neurons. *J. Neurochem.* **73,** 2084–2092.
35. Anderson C. N. G. and Tolkovsky A. M. (1999) A role for MAPK/ERK in sympathetic neuron survival:protection against a p53-dependent, JNK-independent induction of apoptosis by cytosine arabinoside. *J. Neurosci.* **19,** 664–673.
36. Yan C. Y. I. and Greene L. A. (1998) Prevention of PC12 cell death by N-acetylcysteine requires activation of the Ras pathway. *J. Neurosci.* **18,** 4042–4049.
37. Mazzoni I. E., Said F. A., Aloyz R., Miller F. D., and Kaplan D. (1999) Ras regulates sympathetic neuron survival by suppressing the p53-mediated cell death pathway. *J. Neurosci.* **19,** 9716–9727.
38. Hetman M., Kanning K., Cavanaugh J. E., and Xia Z. (1999) Neuroprotection by brain-derived neurotrophic factor (BDNF) is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. *J. Biol. Chem.* **274,** 22,569–22,580.
39. Bonni A., Brunet A., West A. E., Datta S. R., Takasu M. A., and Greenberg M. E. (1999) Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. *Science* **286,** 1358–1362.
40. Schmid R. S., Graff R. D., Schaller M. D., Chen S., Schachner M., Hemperly J. J., and Maness P. F. (1999) NCAM stimulates the Ras-MAPK pathway and CREB phosphorylation in neuronal cells. *J. Neurobiol.* **38,** 542–581.
41. Virdee K. and Tolkovsky A. M. (1995) Activation of p44 and p42 MAP kinases is not essential for the survival of rat sympathetic neurons. *Eur. J. Neurosci.* **7,** 2159–2169.
42. Runden E., Seglen P. O., Haug F. M., Ottersen O. P., Wieloch T., Shamloo M., and Laake J. H. (1998) Regional selective neuronal degeneration after protein phosphatase inhibition in hippocampal slice cultures: evidence for a MAP kinase-dependent mechanism. *J. Neurosci.* **18,** 7296–7305.
43. Park J. A. and Koh J. Y. (1999) Induction of an immediate early gene egr-1 by zinc through extracellular signal-regulated kinase activation in cortical culture: its role in zinc-induced neuronal death. *J. Neurochem.* **73,** 450–456.
44. Stanciu M., Wang Y., Kentor R., Burke N., Watkins S., Kress G., et al. (2000) Persistent activation of ERK contributes to glutamate-induced oxidative toxicity in a neuronal cell line and primary cortical neuron cultures. *J. Biol. Chem.* **275,** 12,200–12,206.
45. Satoh T., Nakatsuka D., Watanabe Y., Nagata I., Kikuchi H., and Namura S. (2000) Neuroprotection by MAPK/ERK kinase inhibition with U0126 against oxidative stress in a mouse neuronal cell line and rat primary cultured cortical neurons. *Neurosci. Lett.* **282,** 163–166.
46. Kirino T. (1982) Delayed neuronal death in the gerbil hippocamus following ischemia. *Brain Res.* **239,** 57–69.
47. Heron A., Pollard H., Dessi F., Moreau J., Lasbennes F., Ben-Ari Y., and Charriaut-Marlangue C. (1993) Regional variability in DNA fragmen-

tation after global ischemia evidenced by combined histological and gel electrophoresis observations in the rat brain. *J. Neurochem.* **61,** 1973–1976.
48. Nitatori T., Sato N., Waguri S., Karasawa Y., Araki H., Shibanai K., Kominami E., and Uchiyama Y. (1995) Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. *J. Neurosci.* **15,** 1001–1011.
49. MacManus J. P., Hill I. E., Preston E., Rasquinha I., Walker T., and Buchan A. M. (1995) Differences in DNA fragmentation following transient cerebral ischemia or decapitation ischemia in rats. *J. Cereb. Blood Flow Metab.* **15,** 728–737.
50. Walton K. M., DiRocco R., Bartlett B. A., Koury E., Marcy V. R., Jarvis B., Schaefer E. M., and Bhat R. V. (1998) Activation of p38MAPK in microglia after ischemia. *J. Neurochem.* **70,** 1764–1767.
51. Gillardon F., Spranger M., Tiesler C., and Hossmann K. A. (1999) Expression of cell death-associated phospho-c-Jun and p53-activated gene 608 in hippocampal CA1 neurons following global ischemia. *Brain Res. Mol. Brain Res.* **73,** 138–143.
52. Sugino T., Nozaki K., Takagi Y., Hattori I., Hashimoto N., Moriguchi T., and Nishida E. (2000) Activation of mitogen-activated protein kinases after transient forebrain ischemia in gerbil hippocampus. *J. Neurosci.* **20,** 4506–4514.
53. Takagi Y., Nozaki K., Sugino T., Hattori I., and Hashimoto N. (2000) Phosphorylation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase after transient forebrain ischemia in mice. *Neurosci. Lett.* **294,** 117–120.
54. Yang D. D., Kuan C. Y., Whitmarsh A. J., Rincon M., Zheng T. S., Davis R. J., Rakic P., and Flavell R. A. (1997) Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature* **389,** 865–870.
55. Ozawa H., Shioda S., Dohi K., Matsumoto H., Mizushima H., Zhou C. J., et al. (1999) Delayed neuronal cell death in the rat hippocampus is mediated by the mitogen-activated protein kinase signal transduction pathway. *Neurosci. Lett.* **262,** 57–60.
56. Hu B. R. and Wieloch T. (1994) Tyrosine phosphorylation and activation of mitogen-activated protein kinase in the rat brain following transient cerebral ischemia. *J. Neurochem.* **62,** 1357–1367.
57. Hu B. R., Liu C. L., and Park D. J. (2000) Alteration of MAP kinase pathways after transient forebrain ischemia. *J. Cereb. Blood Flow Metab.* **20,** 1089–1095.
58. Shioda S., Ozawa H., Dohi K., Mizushima H., Matsumoto K., Nakajo S., et al. (1998) PACAP protects hippocampal neurons against apoptosis:involvement of JNK/SAPK signaling pathway. *Ann. NY Acad. Sci.* **865,** 111–117.
59. Tsuji M., Inanami O., and Kuwabara M. (2000) Neuroprotective effect of alpha-phenyl-N-tert-butylnitrone in gerbil hippocampus is mediated by the mitogen-activated protein kinase pathway and heat shock proteins. *Neurosci. Lett.* **282,** 41–44.
60. Linnik M. D., Zobrist R. H., and Hatfield M. D. (1993) Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. *Stroke* **24,** 2002–2008.
61. Charriault-Marlangue C., Margaill I., Represa A., Popovici T., Plotkine M., and Ben-Ari Y. (1996) Apoptosis and necrosis after reversible focal ischemia: an in situ DNA fragmentation analysis. *J. Cereb. Blood Flow Metab.* **16,** 186–194.
62. Choi D. W. (1996) Ischemia-induced neuronal apoptosis. *Curr. Opin. Neurobiol.* **8,** 667–672.
63. Li Y., Powers C., Jiang N., and Chopp M. (1998) Intact, injured, necrotic and apoptotic cells after focal cerebral ischemia in rat. *J. Neurol. Sci.* **156,** 119–132.
64. Herdegen T., Claret F. X., Kallunki T., Martin-Villalba A., Winter C., Hunter T., and Karin M. (1998) Lasting N-terminal phosphorylation of c-Jun and activation of c-Jun N-terminal kinases after neuronal injury. *J. Neurosci.* **18,** 5124–5135.
65. Martin-Villalba A., Winter C., Brecht S., Buschmann T., Zimmermann M., and Herdegen T. (1998) Rapid and long-lasting suppression of the ATF-2 transcription factor is a common response to neuronal injury. *Brain Res. Mol. Brain Res.* **62,** 158–166.
66. Kitagawa H., Warita H., Sasaki C., Zhang W. R., Sakai K., Shiro Y., et al. (1999) Immunoreactive Akt, PI3-K and ERK protein kinase expression in ischemic rat brain. *Neurosci. Lett.* **274,** 45–48.
67. Irving E. A., Barone F. C., Reith A. D., Hadingham S. J., and Parsons A. A. (2000) Differential activation of MAPK/ERK and p38/SAPK in neurones and glia following focal cerebral ischemia in the rat. *Brain Res. Mol. Brain Res.* **77,** 65–75.
68. Wu D. C., Ye W., Che X. M., and Yang G. Y. (2000) Activation of mitogen-activated protein

kinases after permanent cerebral artery occlusion in mouse brain. *J. Cereb. Blood Flow Metab.* **20,** 1320–1330.
69. Barone F. C. and Feuerstein G. Z. (1999) Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J. Cereb. Blood Flow Metab.* **19,** 819–834.
70. Alessandrin A., Namura S., Moskowitz M. A., and Bonventre J. V. (1999) MEK1 protein kinase inhibition protects against damage resulting from focal cerebral ischemia. *Proc. Natl. Acad. Sci. USA* **96,** 12,866–12,869.
71. Kitagawa K., Matsumoto M., Tagaya M., Hata R., Ueda H., Niinobe M., et al. (1990) 'Ischemic tolerance' phenomenon found in the brain. *Brain Res.* **528,** 21–24.
72. Kirino T., Tsujita Y., and Tamura A. (1991a) Induced tolerance to ischemia in gerbil hippocampal neuron with brief cerebral ischemia on the neuronal damage following secondary ischemic insult in the gerbil: cumulative damage and protective effected neuronal death in the gerbil hippocampus. *Neurosci. Res.* **20,** 95–99.
73. Kirino T., Tsujita Y., and Tamura A. (1991b) Induced tolerance to ischemia in gerbil hippocampal neurons. *J. Cereb. Blood Flow Metab.* **11,** 299–307.
74. Nowak T. S., Jr., Ikeda J., and Nakajima T. (1993) 70 kilodalton heat shock protein and c-fos gene expression as indicators of neuronal pathophysiology after ischemia. *Progr. Brain Res.* **96,** 195–208.
75. Shimazaki K., Ishida A., and Kawai N. (1994) Increase in Bcl-2 oncoprotein and the tolerance to ischemia-induced neuronal death in the gerbil hippocampus. *Neurosci. Res.* **20,** 95–99.
76. Kato H., Kogure K., Araki T., and Itoyama Y. (1995) Induction of Jun-like immunoreactivity in astrocytes in gerbil hippocampus with ischemic tolerance. *Neurosci. Lett.* **189,** 13–16.
77. Heurteaux C., Lauritzen I., Widmann C., and Lazdunski M. (1995) Essential role of adenosine, adenosine A1 receptors, and ATP-sensitive K+ channels in cerebral ischemic preconditioning. *Proc. Natl. Acad. Sci. USA* **92,** 4666–4670.
78. Kawahara N., Ide T., Saito N., Kawai K., and Kirino T. (1998) Propentofylline potentiates induced ischemic tolerance in gerbil hippocampal neurons via adenosine receptor. *J. Cereb. Blood Flow Metab.* **18,** 472–475.
79. Murry C. E., Jennings R. B., and Reimer K. A. (1986) Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **74,** 1124–1136.
80. Weinbrenner C., Liu G.-S., Cohen M. V., and Downey J. M. (1997) Phosphorylation of tyrosine 182 of p38 mitogen-activated protein kinase correlates with the protection of preconditioning in the rabbit heart. *J. Mol. Cell Cardiol.* **29,** 2383–2391.
81. Nagarkatti D. S. and Sha'afi R. I. (1998) Role of p38 MAP kinase in myocardial stress. *J. Mol. Cell Cardiol.* **30,** 1651–1664.
82. Armstrong S. C., Gao W., Lane J. R., and Ganote C. E. (1998) Protein phosphatase inhibitors calyculin A and fostriecin protect rabbit cardiomyocytes in late ischemia. *J. Mol. Cell Cardiol.* **30,** 61–73.
83. Baines C. P., Liu G. S., Birincioglu M., Critz S. D., Cohen M. V., and Downey J. M. (1999) Ischemic preconditioning depends on interaction between mitochondrial KATP channels and actin cytoskeleton. *Am. J. Physiol.* **276,** 1361–1368.
84. Haq S. E. A., Clerk A., and Sugden P. H. (1998) Activation of mitogen-activated protein kinases (p38-MAPKs, SAPKs/JNKs and ERKs) by adenosine in the perfused rat heart. *FEBS Lett.* **434,** 305–308.
85. Sommer C., Gass P., and Kiessling M. (1995) Selective c-JUN expression in CA1 neurons of the gerbil hippocampus during and after acquisition of an ischemia-tolerant state. *Brain Pathol.* **5,** 135–144.
86. Kato H., Kogure K., Araki T., and Itoyama Y. (1995) Induction of Jun-like immunoreactivity in astrocytes in gerbil hippocampus with ischemic tolerance. *Neurosci. Lett.* **189,** 13–16.
87. Sugino T., Nozaki K., and Hashimoto N. (2000) Activation of mitogen-activated protein kinases in gerbil hippocampus with ischemic tolerance induced by 3-nitropropionic acid. *Neurosci. Lett.* **278,** 101–104.
88. Tauskela J. S., Chakravarthy B. R., Murray C. L., Wang Y., Comas T., Hogan M., Hakim A., and Morley P. (1999) Evidence from cultured rat cortical neurons of differences in the mechanism of ischemic preconditioning of brain and heart. *Brain. Res.* **827,** 143–151.
89. Shamloo M., Rytter A., and Wieloch T. (1999) Activation of the extracellular signal-regulated protein kinase cascade in the hippocampal CA1 region in a rat model of global cerebral ischemic preconditioning. *Neuroscience* **93,** 81–88.

90. Gonzalez-Zulueta M., Feldman A. B., Klesse L. J., Kalb R. G., Dillman J. F., Parada L. F., Dawson T. M., and Dawson V. L. (2000) Requirement for nitric oxide activation of p21(ras)/extracellular regulated kinase in neuronal ischemic preconditioning. *Proc. Natl. Acad. Sci. USA* **97,** 436–441.

91. Gu Z., Jiang Q., Zhang G., Cui Z., and Zhu Z. (2000) Diphosphorylation of extracellular signal-regulated kinases and c-Jun N-terminal protein kinases in brain ischemic tolerance in rat. *Brain Res.* **860,** 157–160.