

Signaling pathways mediating anti-apoptotic action of neurotrophins

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Abstract. Neurotrophins promote survival and suppress apoptosis in many populations of neurons. Currently, phosphatidylinositol-3 kinase (PI-3K) is recognized as the main mediator of this protective effect. However, most of the data collected so far on the anti-apoptotic signaling of neurotrophins were obtained using trophic withdrawal paradigms. Recent data from our and other groups indicate that extracellular-signal-regulated kinase 1/2 (Erk1/2) may play a critical role in suppressing neuronal apoptosis triggered by cellular damage. Thus, it appears that either Erk1/2 or PI-3K, depending on the nature of the death-inducing stimulus, can mediate anti-apoptotic signaling of neurotrophins. In this review, we discuss the contribution of Erk1/2 and PI-3K to neuroprotection by neurotrophins. We also present data suggesting possible mechanisms by which these pathways might suppress neuronal death.

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NEUROTROPHINS ANTAGONIZE MANY DIFFERENT FORMS OF APOPTOSIS

Apoptosis was initially described as a morphologically distinctive form of cell death in the developing nervous system. Further studies suggested that neurons, like many other differentiated cells, require the presence of survival factors to suppress the intrinsic cell death machinery and thereby avoid apoptosis (Raff et al. 1993, Pettmann and Henderson 1998). The regulation of apoptosis by survival factors is therefore critical for normal development and proper functioning of the nervous system. In addition, apoptotic-like death was described in injured neurons following such insults as β -amyloid exposure, excitotoxicity, DNA damage, or oxidative stress (Bredesen 1995, Pettmann and Henderson 1998). These observations implicated apoptosis as an important element of several major neurological diseases boosting

the interest in identifying both anti- and pro- apoptotic signal transduction pathways in neurons.

Neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins 3, 4/5 and 6 (NT3, NT4/5, NT6), belong to the first identified group of factors suppressing neuronal apoptosis (Levi-Montalcini and Booker 1960, Barde 1989). These factors interact with two types of receptors characterized by either low or high affinity for neurotrophin binding (Segal and Greenberg 1996, Kaplan 1998, Friedman and Greene 1999, Klesse and Parada 1999). Receptor tyrosine kinases (Trk) A, B and C are high affinity receptors for neurotrophins. Activation of Trks has been implicated in differentiation as well as in suppressing apoptosis (Segal and Greenberg 1996, Kaplan 1998, Friedman and Greene 1999, Klesse and Parada 1999). The protein p75 is a low affinity receptor for neurotrophins which was shown to induce neuronal apoptosis in the absence of Trk signaling (Bredesen and

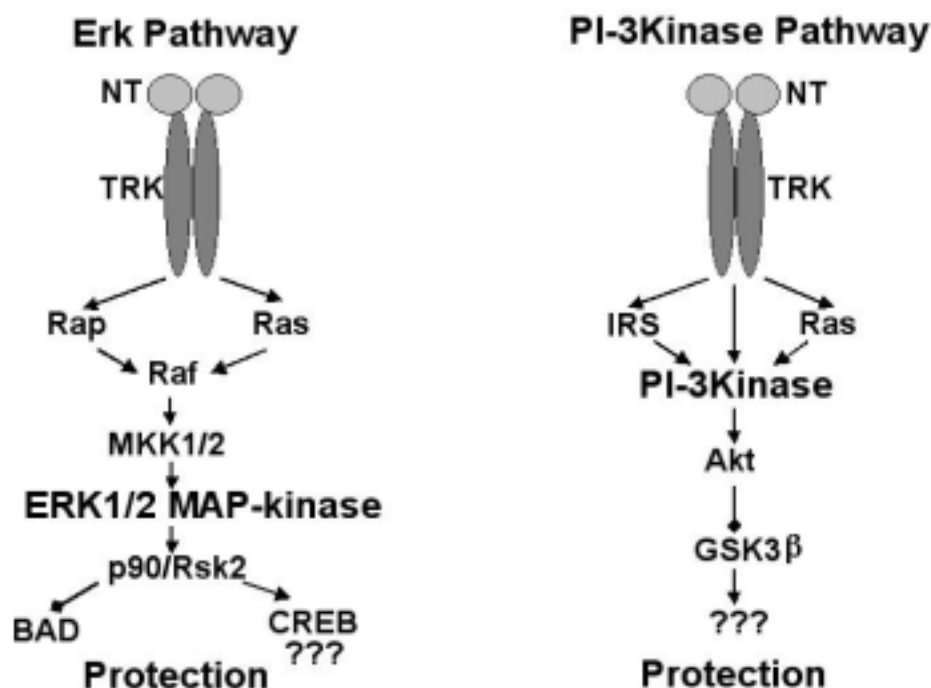


Fig. 1. Erk1/2 and PI-3K pathways as mediators of anti-apoptotic signaling triggered by the interactions of neurotrophins (NT) with their Trk receptors. Arrows and diamonds indicate positive and negative regulation, respectively. Erk1/2 is activated as a consequence of Trk-mediated activation of small GTPases: Ras and Rap. They associate with and activate protein kinase Raf, which phosphorylates and activates MAP kinase kinase 1/2 (MKK1/2). MKK1/2 phosphorylates and activates Erk 1/2, which phosphorylates and activates protein kinase p90/rsk2. This kinase may suppress apoptosis by inhibitory phosphorylation of BAD or activatory phosphorylation of transcription factor CREB. PI-3K can be activated by signaling molecules Ras and IRS1/2 and/or by direct interaction with Trk. Active PI-3K produces lipid second messengers: phosphatidylo-inositol-phosphates which lead to activation of protein kinase Akt. Akt can suppress apoptosis by phosphorylation and inactivation of glycogen synthase kinase-3 β (GSK3 β). See text for more details.

Rabizadeh 1997, Casaccia-Bonnet et al. 1999, Friedman and Greene 1999).

Neurotrophins are able to antagonize several distinct apoptotic stimuli including multiple examples of trophic support deprivation in PNS as well as in CNS neurons (Barde 1989, Ghosh et al. 1994, Silos-Santiago et al. 1995, Yao and Cooper 1995, Bonni et al. 1999, Hetman et al. 1999). In addition to rescuing trophic-deprived neurons, neurotrophins can support survival of nerve cells damaged by a variety of cellular injuries including excitotoxicity, ischemia and multiple neurodegenerative insults (Apfel et al. 1992, Lindholm 1994, Lindsay 1994, Skup 1994, Zheng et al. 1995, Knusel and Gao 1996, Connor and Dragunow 1998, Apfel 1999, Bradford et al. 1999, Hetman et al. 1999). Because apoptosis triggered by either trophic withdrawal or cell damage has many common features including similar morphological alterations, DNA fragmentation and requirement for "killer" gene expression (Raff et al. 1993, Bredesen 1995, Pettmann and Henderson 1998), one could expect that the mechanisms responsible for the neurotrophin mediated protection are also shared.

TRANSDUCTION OF ANTI-APOPTOTIC SIGNALING BY NEUROTROPHINS

As mentioned earlier, anti-apoptotic action of neurotrophins seems to result primarily from their interaction with the high affinity receptors, Trks. Engagement of a Trk receptor by a ligand leads to dimerization of the receptor and activation of multiple signal transduction pathways. Since both the nature of these pathways as well as the mechanism of their activation are the subject of many excellent reviews (Segal and Greenberg 1996, Kaplan 1998, Friedman and Greene 1999, Klesse and Parada 1999) we will focus on the role they play in mediating anti-apoptotic actions of neurotrophins. Activation of the signaling kinases Erk1/2 (Lewis et al. 1998, Cobb 1999, Grewal et al. 1999) and PI-3K (Franke et al. 1997, Hemmings 1997) are some of the events triggered by Trks (Fig. 1). In the middle of the 1990s, Erk1/2 and PI-3K were first implicated in suppressing apoptosis in PC12 cells (Xia et al. 1995, Yao and Cooper 1995). The availability of tools to manipulate various components of these pathways (Table I and II)

Table I

Tools to modify signaling through the Erk1/2 pathway			
Element of the pathway	Available inhibitors	Available activators	Specificity
Ras	dominant negative mutants (Kauffman-Zeh et al. 1997, Klesse and Parada 1998, Mazzoni et al. 1999)	constitutive active mutants (Kauffman-Zeh et al. 1997, Klesse and Parada 1998, Mazzoni et al. 1999)	ras effector mutants thought to be specific (Kauffman-Zeh et al. 1997, Klesse and Parada 1998, Mazzoni et al. 1999)
Raf-1	dominant negative mutants (Klesse and Parada 1998)	constitutive active mutants (Klesse and Parada 1998)	
MKK1/2 (MEK1/2)	dominant negative mutants (Klesse and Parada 1998, Mansour et al. 1994)	constitutive active mutants (Hetman et al. 1999, Klesse and Parada 1998, Mansour et al. 1994)	Thought to be specific
	Drugs: PD98059 (Alessi et al. 1995), SL 327 (Atkins et al. 1998), U0126 (Favata et al. 1998)	unknown	PD98059 directly inhibits cyclooxygenases 1 and 2 (Borsch-Haubold et al. 1998)
Erk1/2	dominant negative mutants (Klesse and Parada 1998)		

Table II

Tools to modify signaling through the PI-3 kinase pathway			
Element of the pathway	Available inhibitors	Available activators	Specificity
Ras	dominant negative mutants (Kauffman-Zeh et al. 1997, Klesse and Parada 1998, Mazzoni et al. 1999)	constitutive active mutants (Kauffman-Zeh et al. 1997, Klesse and Parada 1998, Mazzoni et al. 1999)	ras effector mutants thought to be specific (Kauffman-Zeh et al. 1997, Klesse and Parada 1998, Mazzoni et al. 1999)
PI-3K	dominant negative mutants of p85 regulatory subunit (Dhand et al. 1994, Hetman et al. 1999)	constitutive active mutants of p110 (Hu et al. 1995, Hetman et al. 1999)	Thought to be specific
	Drugs: wortmanin (Arcaro and Wymann 1993), LY294002 (Vlahos et al. 1994)	unknown	unspecific effects reported for wortmanin (Cross et al. 1995, Ferby et al. 1996)
Akt	dominant negative mutants (Dudek et al. 1997)	constitutive active mutants (Dudek et al. 1997, Kauffman-Zeh et al. 1997)	Thought to be specific

led to very rapid accumulation of data on their role in transducing anti-apoptotic signaling of neurotrophins. While most of the data on the involvement of PI-3K were gathered using agents interfering directly with PI-3K activity, the role of the Erk1/2 pathway was usually studied by blocking MAP-kinase kinase 1/2 (MKK1/2 or MEK1/2), an upstream activator of Erk1/2, controlling its activity by phosphorylation. However, MKK1 may also play other roles than controlling Erk activity (Alessandrini et al. 1996). In addition, Erk may also be controlled by MKK1-independent mechanisms (Grammer and Blenis 1997). Therefore some caution must be taken while interpreting effects of MKK1 inhibition as evidence of Erk involvement. Finally, PI-3K might also affect Erk1/2 activity (Lopez-Illasaca et al. 1997, Perkinton et al. 1999), so PI-3K and Erk1/2 may constitute one anti-apoptotic signaling circuit.

ROLE OF PI-3K IN SUPPRESSING NEURONAL APOPTOSIS BY NEUROTROPHINS

Currently, activation of PI-3K is one of the most widely recognized anti-apoptotic signaling events. The first report on the requirement of the PI-3K pathway for protection from apoptosis came in 1995 by Yao and

Cooper, who discovered that this molecule is necessary for NGF, EGF, insulin and serum mediated survival of PC12 cells (Yao and Cooper 1995). In this study, protection provided for serum-starved PC12 cells by NGF, EGF and insulin was unaffected by expressing a RasN17 mutant, which blocked activation of Erk1/2 pathway. In contrast, wortmanin, a PI-3K inhibitor potently inhibited the protection provided by these factors. This drug also induced apoptosis in serum maintained cells. To provide more evidence for PI-3K involvement in suppressing apoptosis, PC12 cells were transfected with the PDGF receptor. It enabled PDGF to protect PC12 cells against serum starvation. Expression of a mutant form of the PDGF receptor, unable to activate PI-3K but retaining the ability to activate Erk1/2, produced cells that were unresponsive to the anti-apoptotic action of PDGF.

Following these initial observations, the PI-3K requirement for neurotrophin produced suppression of apoptosis has been further confirmed. For example, NGF-mediated survival was shown to be transduced by PI-3K in PC12 cells (Klesse et al. 1999) as well as cultured SCG and DRG neurons (Crowder and Freeman 1998, Klesse and Parada 1998, Meyer-Franke et al. 1998, Mazzoni et al. 1999, Vaillant et al. 1999). Furthermore, BDNF protection of serum starved neuroblastoma

cells was sensitive to PI-3K inhibitors (Encinas et al. 1999). Interestingly, PI-3K activation and survival were shown to be independent of Ras in PC12 cells (Yao and Cooper 1995, Klesse et al. 1999) but not in SCG or DRG neurons (Klesse and Parada 1998, Mazzoni et al. 1999, Vaillant et al. 1999). In addition to multiple papers supporting the role of PI-3K in mediating survival promoting effects of neurotrophins in trophic deprived PNS neurons, there are some recent reports questioning its importance (Virdee et al. 1999).

The role of PI-3K in mediating anti-apoptotic action of neurotrophins has also become evident in the CNS. In cerebellar granule cells deprived of trophic support by potassium depolarization, wortmanin blocked BDNF-mediated suppression of apoptosis (Shimoke et al. 1997). Also, in spinal cord motoneurons, BDNF promoted survival through the PI-3K pathway (Dolcet et al. 1999). However, in trophic deprived retinal ganglionic cells treated with BDNF and forskolin, PI-3K was found to be dispensable for protection from cell death (Meyer-Franke et al. 1998). In another study with cerebellar granule cells protected from oxidizing stress by BDNF, PI-3K was found to be partially involved (Skaper et al. 1998). Similar partial involvement of PI-3K was shown in BDNF-mediated protection against ionomycin induced apoptosis in cortical neurons (Takei et al. 1999). Interestingly, both Skaper et al. (1998) and Takei et al. (1999) found that the protection afforded by BDNF was also partially dependent on Erk1/2.

In our lab, we addressed the issue of the role of PI-3K in transducing anti-apoptotic actions of neurotrophins in cultured cortical neurons from newborn rats. We and others have shown that this neuronal population responds to BDNF by activation of both PI-3K and Erk 1/2 (Ghosh and Greenberg 1995, Yamada et al. 1997, Hetman et al. 1999). Using these cells, we established a paradigm of trophic deprivation-induced apoptosis by removing serum from the culture media at day 5-7 after plating (Hetman et al. 1999). BDNF-mediated protection against this apoptosis was completely abolished by the PI-3K inhibitor, LY294002 (Hetman et al. 1999). Taking advantage of the calcium-phosphate transfection technique of CNS neurons, we have also shown that transient expression of both wild type and active mutants of PI-3K provides full protection from trophic withdrawal-induced death (Hetman et al. 1999). Our data suggest that PI-3K is both necessary and sufficient for BDNF mediated protection of serum withdrawn cortical neurons.

We also examined the mechanism by which BDNF protected neurons from DNA damage-induced apoptosis. This type of death was induced by exposing primary cortical neurons to camptothecin (CPT), a topoisomerase I inhibitor which causes extensive neuronal apoptosis (Morris and Geller 1996). It is important to note that DNA-damage is postulated to contribute to neuronal death in neurodegenerative diseases (Cotman and Su 1996). Therefore, identifying pathways which protect against DNA damage-induced neuronal death might provide valuable information leading towards effective neuroprotective therapy. We found that BDNF mediated protection from CPT-induced death was not sensitive to LY294002 (Hetman et al. 1999). Consistently, transient expression of PI-3K did not protect from this form of apoptosis (Hetman and Xia, unpublished observation). These data suggest that BDNF-mediated protection of CPT-damaged neurons is not dependent on PI-3K.

PI-3K-MEDIATED PROTECTION BY AGENTS OTHER THAN NEUROTROPHINS

In addition to neurotrophins, there are many other factors supporting survival of neurons and preventing or slowing down apoptosis. Transduction of the anti-apoptotic trophic support signals exerted by these factors frequently involves the PI-3K pathway. For example, PI-3K activation has been shown to be required for IGF-1-mediated survival of cerebellar granule neurons, oligodendrocytes, and PC12 cells (Yao and Cooper 1995, Vemuri and McMorris 1996, D'Mello et al. 1997, Miller et al. 1997, Parrizas et al. 1997). NMDA- and membrane depolarization-mediated survival of cerebellar granule and SCG neurons was also shown to depend on PI-3 kinase activity (Miller et al. 1997, Shimoke et al. 1997, Zhang et al. 1998, Crowder and Freeman 1999). Another example of PI-3K dependent suppression of apoptotic death is protection of trophic deprived motoneurons by glia derived neurotrophic factor (GDNF) (Soler et al. 1999). Midkine, a member of the heparin-binding neurotrophic factors family, produced protection of serum-deprived cortical neurons, which was inhibited by both PI-3K inhibitor, LY294002 and MKK1/2 inhibitor PD98059 (Owada et al. 1999). In this case, PI-3K was suggested to exert its protective action by activating Erk1/2. Interestingly, serum, which is used in many *in vitro* preparations of nerve cells, also sup-

presses apoptosis by activating PI-3K. PI-3K involvement in serum action was observed in a variety of neuronal or neuronal-like cells, including PC12 cells and cortical neurons (Yao and Cooper 1995, Hetman et al. 1999). Since data from our lab excluded Erk1/2 as a transducer of serum mediated suppression of apoptosis in cortical neurons (Hetman et al. 1999), PI-3K seems to be the major mediator of serum actions on cortical neuron survival. Although it is generally accepted that PI-3K pathway is anti-apoptotic, there are reports that it might mediate potentiation of necrotic neuronal death by such factors as insulin or IGF1 (Ryu et al. 1999).

ROLE OF ERK1/2 IN SUPPRESSING NEURONAL APOPTOSIS BY NEUROTROPHINS

As in the case of PI-3K, the first observation suggesting anti-apoptotic action of Erk1/2 signaling pathway was done in PC12 cells (Xia et al. 1995). Following neuronal differentiation by NGF, survival of these cells became dependent on the presence of the neurotrophin. NGF withdrawal-induced apoptosis was prevented by overexpression of activated mutants of MKK1 indicating that the Erk1/2 pathway is sufficient to protect trophic-deprived cells. The fundamental difference between these findings and the findings of Yao and Cooper, who excluded Erk involvement in promoting PC12 survival, can be explained by the usage of non-differentiated, dividing PC12 cells in their study. In addition to differentiated PC12 cells, trophic withdrawn retinal ganglia cells and cerebellar granule neurons were protected by BDNF through the Erk1/2 pathway (Meyer-Franke et al. 1998, Bonni et al. 1999). However, other studies using both pharmacological inhibitors and various mutants of the Erk1/2 pathway have suggested that ERK is not the major mediator of the neuroprotection afforded by neurotrophins, IGF-1 or membrane depolarization in PC12, SCG, DRG or cerebellar neurons following trophic withdrawal (Creedon et al. 1996, Virdee and Tolkovsky 1996, Gunn Moore et al. 1997, Miller et al. 1997, Klesse and Parada 1998, Klesse et al. 1999, Mazzoni et al. 1999). Therefore, the role of Erk1/2 in mediating anti-apoptotic effects of neuro-trophins is still controversial. This is in contrast to the widely accepted contribution of Erk1/2 to transduction of differentiation signals delivered by neurotrophins (Kaplan 1998, Klesse and Parada 1998, Klesse et al. 1999, Mazzoni et al. 1999) or its recognized role in neuronal plasticity (Impey et al. 1999).

In our laboratory, we addressed the issue of Erk1/2 involvement in mediating the anti-apoptotic action of neurotrophins in rat cortical neurons (Hetman et al. 1999). Consistent with the findings excluding the Erk1/2 pathway as a major mediator of trophic support signaling, PD98059, a specific inhibitor of MKK1/2 was unable to affect BDNF-mediated protection of serum starved cells. Similarly, overexpression of an activated mutant of MKK1 did not provide any protection against serum withdrawal. However, in the case of CPT-induced death, BDNF protection was completely abolished by PD98059. Also, overexpression of the activated MKK1 mutant effectively protected CPT-treated neurons (Hetman et al. 1999) (Fig. 2). These data suggest that Erk1/2 is both necessary and sufficient for BDNF-mediated protection from CPT induced apoptosis. Similarly, in SCG neurons exposed to a DNA-damaging agent, cyto-

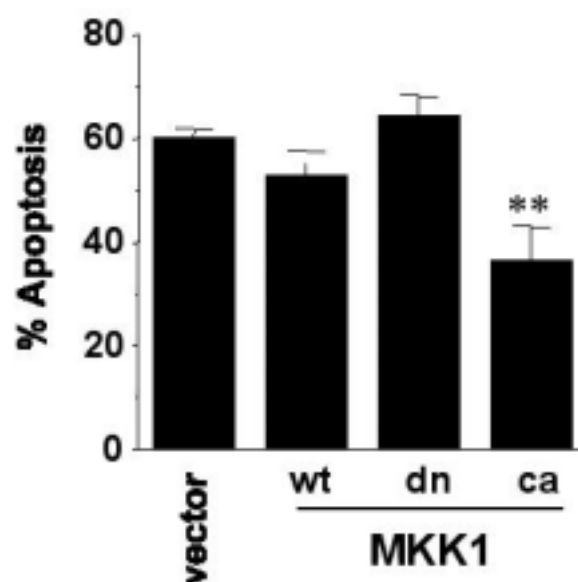


Fig. 2. Cultured newborn rat cortical neurons were transfected as described (Hetman et al. 1999) with various forms of MKK1 including wild type (wt), dominant negative (dn) and constitutive active (ca) (Mansour et al. 1994). Four μ g of plasmid DNA were used in each case. To detect transfected cells 2 μ g of expression plasmid for bacterial beta-galactosidase were cotransfected. Two days after transfection camptothecin (10 μ M) was added for 24 h. Percentage of apoptosis was determined in transfected cells detected by beta-galactosidase immunostaining and counterstained with bisbenzimidazole to visualize apoptotic alterations in nuclear morphology (Hetman et al. 1999). Averages of duplicate determinations in 4 independent experiments are shown. Error bars depict SEM. (**), $P < 0.01$, ANOVA.

sine arabinoside, Erk1/2 turned out to be necessary for NGF mediated protection from apoptosis (Anderson 1999). However, there was a report suggesting no Erk1/2 involvement in the protective action of NGF against UV-induced apoptosis in *trkA*-expressing fibroblasts (Ulrich et al. 1998). Apoptosis induced by other forms of injury was also attenuated by BDNF, at least in part through Erk 1/2, including oxidative damage in cerebellar granule cells (Skaper et al. 1998) and ionomycin treatment of cortical neurons (Takei et al. 1999).

ERK1/2-MEDIATED PROTECTION BY AGENTS OTHER THAN NEUROTROPHINS

In cerebellar granule cells Erk1/2 activation by cAMP was implicated in mediating the anti-apoptotic action of pituitary adenylyl cyclase activating peptide (PACAP) (Villalba and Journot 1997). Activation of Erk1/2 was also shown to be responsible for the neuroprotective actions of estrogen in glutamate challenged neurons (Singer et al. 1999). Protection of serum deprived cortical neurons by midkine was suggested to require PI-3K dependent activation of Erk1/2 (Owada et al. 1999). Suppression of serum deprivation induced death of the neuron-like Gn10 GnRH cells by Gas6, a ligand for receptor tyrosine kinase Ark was dependent on the Erk1/2 pathway (Allen et al. 1999). Rosveratrol, a neuro-protective anti-oxidant from wine, was suggested to protect neurons by activating the Erk1/2 pathway (Tredici et al. 1999). Another compound activating the Raf1/Erk pathway is SB203358, an established inhibitor of p38 MAP kinase (Kalmes et al. 1999). Since this drug and its relatives have been widely used to show a p38-dependent component of neuronal death (Behrens et al. 1999, Castagne and Clarke 1999, Harada and Sugimoto 1999), its Erk-activating properties must be taken into account while interpreting these results.

Intriguingly, damaging stimuli such as camptothecin or glutamate seem to activate Erk1/2 in cortical neurons (Hetman et al. 1999, Gonzalez-Zulueta et al. 2000). Since addition of PD98059 accelerated CPT-triggered cell death, Erk1/2 activation was implicated as a defense mechanism against injury in that system (Hetman et al. 1999). Induction of ischemic tolerance by exposure to transient ischemia episodes was shown to be dependent on the NMDA receptor-driven NO/Ras/Erk1/2 pathway (Gonzalez-Zulueta et al. 2000). Furthermore, in several non-neuronal systems damaging stimuli such as oxida-

tive stress, radiation or Fas ligand were shown to mobilize anti-apoptotic Erk activity (Gardner and Johnson 1996, Guyton et al. 1996, Carter et al. 1998, Holmstrom et al. 1999). These data suggest an interesting possibility that Erk1/2 activation might be a general defense mechanism to protect different cells types against various forms of damage. There are several reports, however, implicating MKK1/Erk activation as an important component of the neuronal death cascade following damaging stimuli such as focal cerebral ischemia (Alessandrini et al. 1999), seizures (Murray et al. 1998), zinc (Park and Koh 1999) and okadaic acid (Runden et al. 1998).

It seems that both PI-3K and Erk 1/2 might transduce anti-apoptotic signals by neurotrophins. The importance of these two pathways in neuroprotection is dependent on the cell type as well as on the nature of the apoptotic stimulus. For example, in cortical and sympathetic neurons PI-3K appears to be necessary for protecting from trophic withdrawal while Erk1/2 is critical to protect from DNA damage. Data from other neuronal populations, although incomplete, would also support the idea of PI-3K as a critical trophic support pathway and of Erk1/2 as a protector against damage-induced apoptosis.

MECHANISMS OF PI-3K MEDIATED PROTECTION

Recently, intense efforts of many researchers shed some light on the mechanisms by which the PI-3K pathway inhibits apoptosis. It seems that the protein kinase Akt (also known as PKB or RAC) may mediate cellular survival due to activation of PI-3 kinase (Dudek et al. 1997, Kauffmann-Zeh et al. 1997, Kulik et al. 1997, Philpott et al. 1997, Crowder and Freeman 1998). In addition to multiple non-neuronal cells, importance of Akt has been demonstrated in cerebellar granule neurons, SCG, DRG neurons, neuronal cell lines such as PC12 and neuroblastoma (Dudek et al. 1997, Philpott et al. 1997, Crowder and Freeman 1998, Pap and Cooper 1998, Yano et al. 1998). It is important to note that direct evidence linking anti-apoptotic action of PI-3K activated by neurotrophins to Akt is missing in primary CNS neurons. Moreover, there are reports that Akt can promote survival independently of PI-3K activity (Yano et al. 1998, Virdee et al. 1999). This is in concert with biochemical data suggesting that such anti-apoptotic second messengers as calcium or cAMP can drive Akt activity in a PI-3K independent manner (Yano et al.

1998, Filippa et al. 1999). Neurotrophin-activated Akt was implicated in survival of SCG neurons and in neuron-like PC12 cells (Crowder and Freeman 1998, Pap and Cooper 1998, Vaillant et al. 1999, Virdee et al. 1999). There were, however, reports questioning the role of Akt in NGF mediated survival of SCG neurons (Philpott et al. 1997).

Akt phosphorylates multiple substrates including proteins regulating apoptosis (Datta et al. 1999) (Fig. 3). The pro-apoptotic protein Bad was identified as Akt's first apoptosis-relevant substrate (Datta et al. 1997, del Peso et al. 1997). Bad phosphorylation at serine 136 inhibits its apoptosis-inducing properties. Human (Cardone et al. 1998), but not mouse (Fujita et al. 1999), caspase 9 is also subject to inhibitory phosphorylation by Akt. Other identified targets for anti-apoptotic phosphorylation by Akt include transcription factor FKHRL1 (Brunet et al. 1999), NF κ B activating kinase IKK1 α (Ozes et al. 1999, Romashkova and Makarov 1999), L-type Ca²⁺ channels (Blair et al. 1999) and glycogen synthase kinase-3 β (GSK3 β) (Pap and Cooper 1998, Hetman et al. 2000). In the case of FKHRL1, it was postulated that Akt inhibits FKHRL1-mediated Fas ligand transcription thereby suppressing apoptosis (Brunet et al. 1999). The recently shown activation of nitric oxide

synthase by Akt-mediated phosphorylation might also potentially contribute to survival (Dimmeler et al. 1999, Fulton et al. 1999). Obviously, these anti-apoptotic mechanisms might operate simultaneously in one cell or they might be cell-type or species-specific. So far Bad, FKHRL1, L-type calcium channels and GSK3 β have been implicated as anti-apoptotic targets for Akt in neuronal or neuronal-like systems. Of those, only GSK3 β was linked to neurotrophin-mediated activation of the PI-3K/Akt pathway.

In PC12 cells and in primary cortical neurons, GSK3 β was activated upon trophic withdrawal and suppressed by adding NGF or BDNF, respectively (Pap and Cooper 1998, Hetman et al. 2000). Inhibiting the kinase by overexpressing either a GSK3 β binding protein which is a specific GSK3 β inhibitor, or a dominant negative GSK3 β protected against trophic withdrawal induced apoptosis (Pap and Cooper 1998, Hetman et al. 2000). Moreover, BDNF was able to suppress apoptosis induced by overexpressed GSK3 β (Hetman and Xia, unpublished observation). The mechanism for induction of apoptosis by GSK3 β remains undefined. GSK3 β phosphorylates four serine residues at the amino-terminal region of β -catenin and causes β -catenin degradation (Miller and Moon 1996, Yost et al. 1996). It has been

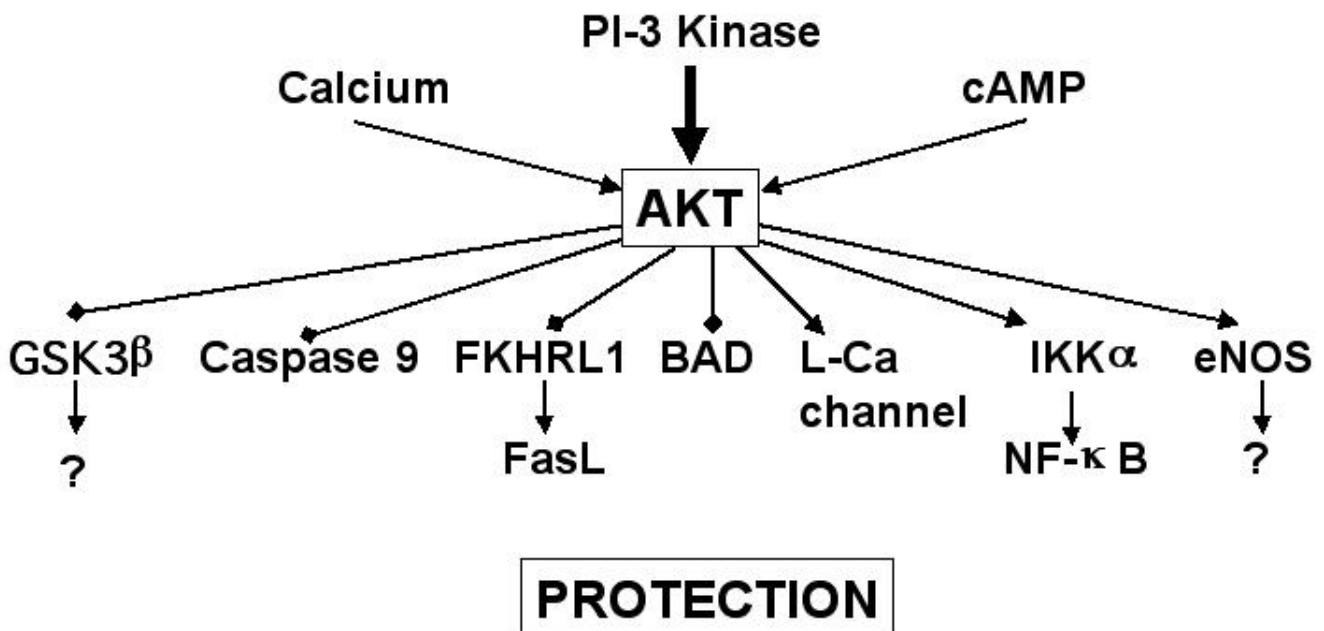


Fig. 3. Anti-apoptotic targets for PI-3K/Akt pathway. Arrows and diamonds indicate positive and negative regulation, respectively. See text for details.

proposed that destabilization of β -catenin potentiates neuronal apoptosis induced by β -amyloid peptide (Zhang et al. 1998). However, data from our lab do not support β -catenin destabilization as a mechanism of GSK3 β -mediated apoptosis because over-expression of wild type or a stable mutant form of β -catenin did not rescue cortical neurons from trophic deprivation-induced death (Hetman et al. 2000). In addition to β -catenin and glycogen synthase, several other substrates for GSK3 β have been identified which may be candidate mediators of GSK3 β -induced cell death. Examples of such substrates include pyruvate dehydrogenase (Hoshi et al. 1996), insulin receptor substrate 1 (IRS-1) (Eldar-Finkelman and Krebs 1997), and microtubule associated protein tau (Hanger et al. 1992, Mandelkow et al. 1992).

It is important to note that some of the established targets for Akt signaling may be regulated by alternative routes. For example, in the lymphoid progenitor cells FL5.12, interleukin 3-mediated survival was shown to be dependent on PI-3K-regulated activation of p21 activated kinase 1 (PAK1) (Schurmann et al. 2000). PAK1 mediated phosphorylation of serines 112 and 136 of Bad was implicated as a mechanism for this protection. Interestingly, it was suggested that PI-3K mediated activation of the transcription factor NF κ B might contribute to neuroprotective action of IGF1 in neuronal GT1-7 cells challenged with hydrogen peroxide (Heck et al. 1999). Although it is well established that PI-3K activity is crucial for many anti-apoptotic effects of neurotrophins, the downstream events are obscure and require further studies.

MECHANISMS OF ERK MEDIATED PROTECTION

Downstream events of Erk1/2 activation that are crucial for its ability to suppress apoptosis are less well understood than those of the PI-3K pathway. Recently there has been a report suggesting that Erk1/2 activation by BDNF suppressed apoptosis in trophic deprived cerebellar granule cells by activating the serine/threonine protein kinase p90/rsk2 (Bonni et al. 1999). The pro-apoptotic protein Bad and transcription factor CREB were implicated as p90/rsk2 substrates important to suppress apoptosis (Bonni et al. 1999). Bad phosphorylation at serine 112 by rsk2 had similar inhibitory effects as serine 136 phosphorylation by Akt. Activating phosphorylation of CREB at serine 133 was suggested to

increase expression of survival genes. In another report CREB inhibition by the expression of dominant negative mutants led to the death of SCG neurons maintained in the presence of NGF (Riccio et al. 1999). Moreover, the mechanism by which CREB suppressed apoptosis was suggested to be NGF-induced and CREB-mediated upregulation of anti-apoptotic protein bcl-2 (Riccio et al. 1999). However, a direct link between CREB and Erk is missing in both SCG and cerebellar granule cells. It is very important to explore this connection, especially since CREB might also be activated by other mediators of neuroprotective signaling such as Akt or cAMP (Montminy 1997, Du and Montminy 1998). It is interesting to note that in *Drosophila*, activation of Erk is able to inhibit apoptosis by negative regulation of an activator of caspases, hid (Bergmann et al. 1998, Kurada and White 1998). It is possible that analogous mechanism also exists in mammals.

OTHER PROTECTIVE PATHWAYS CONTROLLED BY NEUROTROPHINS

In addition to Erk1/2 and PI-3K pathways there are many more signaling circuits regulated by neurotrophins such as phospholipase C γ (PLC γ), small GTPases ras and rho, protein kinases C, p38, Erk5, Jnk, transcription factor NF κ B and tyrosine phosphatase Shp (Segal and Greenberg 1996, Kamakura et al. 1999, Kaplan 1998, Xing et al. 1998). Some of them including PLC γ , protein kinase C or protein kinase p38, a relative of Erk1/2, were implicated in survival responses to such stimuli as NGF, serum, phorbol esters or membrane depolarization (Ulrich et al. 1998, Behrens et al. 1999, Mao et al. 1999). Interestingly, in SCG cells, NF κ B was found to be necessary for the anti-apoptotic action of NGF (Maggirwar et al. 1998). One should also emphasize that although the p75 receptor for neurotrophins is generally considered to be pro-apoptotic, it can activate a protective transcription factor, NF κ B (Hamanoue et al. 1999). It remains to be determined how general the roles of PLC γ , PKC, p38 or NF κ B are in transducing anti-apoptotic actions of neurotrophins.

CONCLUSIONS AND PERSPECTIVE

Collectively, the reviewed literature as well as data from our laboratory would suggest that the major pathways transducing the anti-apoptotic effects of neuro-

trophins are PI-3K and Erk1/2. The nature of the apoptotic stimulus seems to be a critical determinant of pathway preference. Thus, it appears that in most cases of trophic deprivation, PI-3K is the main player, whereas Erk1/2 dominates as a major neuroprotective mechanism in damaged cells. The main challenge in the field is to learn how these pathways protect neurons. This knowledge may lead to new strategies for effective neuroprotection in diseases. For example, GSK3 β is an emerging candidate drug target for neuroprotection, which was identified as a substrate for PI-3K mediated suppression of apoptosis.

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