Amino Acids (2002) 23: 11–17
DOI 10.1007/s00726-001-0103-9

Amino
Acids
Printed in Austria

Co-activation of the phosphatidylinositol-3-kinase/Akt signaling pathway by N-methyl-D-aspartate and TrkB receptors in cerebellar granule cell neurons

D. Zhu[1], **R. H. Lipsky**[2], and **A. M. Marini**[1]

[1] Department of Neurology and Neuroscience, Uniformed Services University of the Health Sciences, Bethesda, Maryland, U.S.A.
[2] Section on Molecular Genetics, Laboratory of Neurogenetics, NIAAA, National Institutes of Health, Rockville, Maryland, U.S.A.

Received June 29, 2001
Accepted August 6, 2001
Published online June 3, 2002

Summary. Neuroprotective concentrations of N-methyl-D-aspartate (NMDA) promote survival of cerebellar granule cell neurons against glutamate excitotoxicity through a TrkB receptor-mediated brain-derived neurotrophic factor (BDNF) autocrine loop. However, the intracellular signaling pathway(s) are not clear. Our results show that PI-3 kinase/Akt is activated by either NMDA or BDNF displaying differential kinetics. BDNF and NMDA increased Akt phosphorylation within 5 minutes but maximal activation by NMDA was observed at 3 hours. Akt phosphorylation was completely blocked by the PI-3 kinase inhibitor LY294002. NMDA-mediated activation of Akt was completely blocked by MK-801 and partially blocked by the TrkB receptor inhibitor, K252a, indicating the requirement of TrkB receptors for maximal activation by NMDA. In contrast, BDNF-induced Akt phosphorylation was abolished by K252a, but not by the addition of MK-801. Therefore, the PI-3 kinase/Akt pathway is co-activated by NMDA and TrkB receptors. The kinetics of BDNF and NMDA-mediated activation of PI-3 kinase/Akt suggests that they have different roles in intraneuronal time-related events.

Keywords: N-methyl-D-aspartate – Brain-derived neurotrophic factor – Rat cerebellar granule cells – Phosphatidylinositol 3-kinase/Akt – TrkB receptor – Neuroprotection

Introduction

Glutamate, the endogenous excitatory neurotransmitter, is also involved in the pathophysiology of hypoxic/ischemic neuronal injury (Olney et al., 1971; Wieloch, 1985; Choi, 1988). Among all the glutamate receptors, ie, ionotropic receptors and G-protein-coupled metabotropic receptors, the N-methyl-D-aspartate (NMDA) receptor is thought to play a major role in this neuropathological process (Koh and Choi, 1988a, 1988b).

Cerebellar granule cells, the most abundant neuronal subtype in the mammalian brain, have been used extensively for studying the molecular mechanisms underlying neuronal death and survival. Studies have shown that overactivation of NMDA receptors results in neuronal cell death in rat cerebellar granule cells (Favaron et al., 1988; Novelli et al., 1988; Schramm et al., 1990). Paradoxically, subtoxic concentrations of NMDA are neuroprotective against an excitotoxic concentration of glutamate (Marini et al., 1992; Marini et al., 1998; Lipsky et al., 2001). We observed that preincubation with subtoxic concentrations of NMDA reduced the toxicity mediated by glutamate, and the neuroprotective effect was blocked by either RNA or protein synthesis inhibitors (Marini et al., 1992). This indicates that NMDA neuroprotection may involve a neuroprotective polypeptide.

Cerebellar granule cells are responsive to various neurotrophins, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), etc, whose biological activity depends upon the activation of high-affinity receptors (Trk) (Lindholm et al., 1993; Segal et al., 1995; Rodriguez-Tebar et al., 1990; Lamballe et al., 1991).

Our recent data showed that activation of NMDA receptors results in the release of BDNF which in turn binds to and activates its cognate receptor, TrkB, leading to the first and necessary step in transducing signals to protect neurons against glutamate-induced excitotoxicity. By increasing BDNF release and activating TrkB receptors, NMDA therefore indirectly exerts neurotrophic activity (Marini et al., 1998). However, the intracellular signal transduction pathways involved in the neurotrophic activity are still not clear.

The fate of neurons depends on the balance between "positive" signal transduction cascades that actively promote survival and "negative" cascades enhancing apoptosis (Williams et al., 1999). Phosphoinositide-3 kinase (PI-3 kinase)/Akt signaling pathway has been shown to play an important role in neuroprotection in some systems and cell types (Dudek et al., 1997; Blair et al., 1999; Kim et al., 2000; Ng et al., 2000; Zhang et al., 2000). In this paper, we report that maximal activation of the PI-3 kinase/Akt signaling pathway by neuroprotective concentrations of NMDA requires both NMDA and TrkB receptors in cerebellar granule neurons.

Materials and methods

Materials

NMDA was purchased from Cambridge Research Biochemicals (Harston, U.K.). Human recombinant BDNF was from Promega (Madison, WI). Phosphoplus PI-3 kinase/Akt western blot kit was from Cell Signaling (Beverly, MA). PI-3 kinase inhibitor, LY294002 was from Calbiochem (La Jolla, CA). MK-801 and K252a were obtained from Research Biochemicals (Natick, MA). Cell culture media and supplies were purchased from Life Technologies, Inc. (Rockville, MD). All other chemicals were obtained from Sigma Chemical Corp (St. Louis, MO).

Cell culture

Granule cells were prepared from postnatal day 8 Sprague Dawley rat pups. Briefly, meninges-free cerebella were minced and recovered by centrifugation. The pellets from cerebella were subjected to trypsinization, followed by inactivation of trypsin by the addition of soybean trypsin inhibitor. Cells were then dissociated by a series of triturations and recovered by centrifugation. The final pellet was reconstituted in Basal Eagle's medium containing glutamine (2 mM), fetal calf serum (10%), and potassium chloride (25 mM). No antibiotics were added, and the plating density was 1.8×10^6 cells/ml. Cytosine arabinoside (10 μM) was added 18–24 h later to inhibit the proliferation of non-neuronal constituents. On day 7 in vitro, glucose (100 μl of a 100 mM solution) and sterile water (100 μl) were added to each 35-mm culture dish to maintain survival and to replace evaporative losses, respectively (Marini et al., 1998).

Treatment of cells

All experiments were carried out with cultured cerebellar granule cells on DIV 8. To assess the effect of NMDA and BDNF on the activation of PI-3 kinase/Akt pathway, NMDA (100 μM) or BDNF (100 ng/ml) was added to the culture medium for the indicated time period before the preparation of cell lysates (see Western blot analysis). In our previous study, we demonstrated that NMDA (100 μM) is subtoxic and has the maximal neuroprotection against glutamate toxicity and BDNF (100 ng/ml) exhibits a comparable neuroprotective effect. For inhibition experiments, cerebellar granule cells were pretreated with or without MK-801 (1 μM), K252a (10 nM), LY294002 (10 μM) for 1 hour prior to the addition of NMDA (100 μM) or BDNF (100 ng/ml).

Western blot analysis (Akt phosphorylation)

Cultured cerebellar granule cells were harvested in 200 μl of cell lysis buffer (1% Nonidet P-40, 20 mM Tris-HCl, pH 7.4, 137 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonylfluoride, 0.15 units/ml aprotinin, 20 μM leupeptin, 1 mM sodium vanadate). After removal of cellular debris by centrifugation, protein levels in the lysates were measured by the Bio-Rad protein assay method and equalized accordingly. After polyacrylamide gel electrophoresis separation, proteins were transferred onto nitrocellulose membrane. The membrane was blocked for 1 hour at room temperature in blocking buffer (2% bovine serum albumin, 10 mM Tris-HCl pH 8.0, 0.15 M NaCl, 0.2% Tween 20), followed by incubation overnight at 4°C with anti-phospho-Akt (Ser473) antibody [1 : 1000 dilution in Tris-buffered saline/0.2% Tween 20 (TBST)]. After washing in TBST, the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit antibody (1 : 2000 dilution) for 1 hour at room temperature. Immunoreactive bands were visualized using the enhanced chemiluminescence method. The membrane was stripped and reblotted with the non-phospho-Akt antibody for the detection of the total protein level of Akt.

Results

The effect of NMDA and BDNF on PI-3 kinase activity was determined by Akt phosphorylation which is a direct downstream target of PI-3 kinase (Datta et al., 1996; Franke et al., 1997). We assessed the kinetics of Akt activation by NMDA and BDNF by determining the kinetics of phosphorylation occurring at residue Ser473 which is required for maximal Akt catalytic activity.

Activation of PI-3 kinase/Akt pathway by NMDA

Incubation of cerebellar granule neurons with 100 μM NMDA increased the phosphorylation of Akt in a time-dependent manner with the maximal occurring at 3 hours (Fig. 1A). Interestingly, there is a small increase in Akt phosphorylation by NMDA at 5 min when compared with the total protein levels of Akt. Preincubation of LY294002, the PI-3 kinase inhibitor, prior to the addition of NMDA, completely blocked NMDA-mediated activation of Akt (Fig. 1B).

Activation of PI-3 kinase/Akt pathway by BDNF

In contrast to the activation of Akt by a maximum neuroprotective concentration of NMDA, Akt is rapidly activated by BDNF (100 ng/ml) within 5 minutes (Fig. 2A). This result is in accordance with the NMDA data where a smaller increase in Akt phosphorylation was observed possibly due to the lower BDNF concentration in NMDA-treated neurons. Phosphoryla-

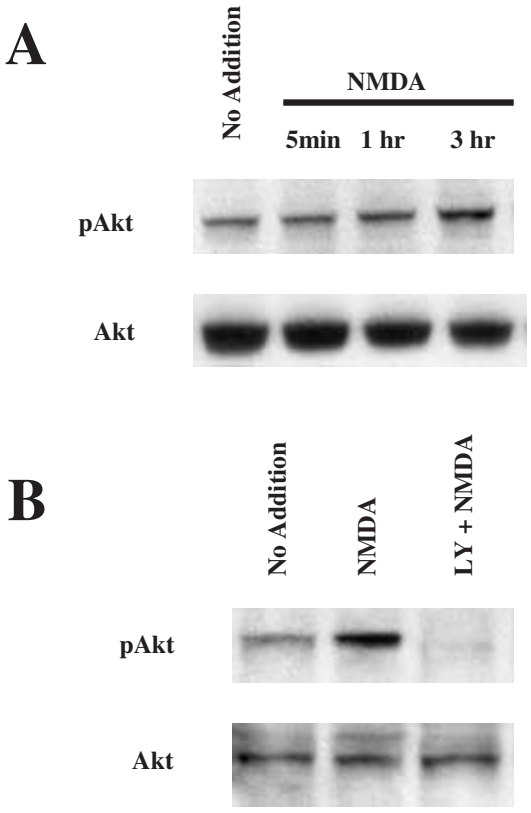

Fig. 1. Effect of NMDA on PI-3 kinase/Akt activation. **A** Cells were treated with medium alone (cont) or 100 μM NMDA for 5 minutes, 1 hour and 3 hours. Neuronal cell lysates were prepared and subjected to western blot analysis as described in Materials and Methods. **B** Cells were incubated with medium alone (cont), 100 μM NMDA (NMDA), or 10 μM LY294002 plus NMDA (LY + NMDA) for 3 hour. Cells were exposed to LY294002 for 1 hour prior to the addition of NMDA. pAkt indicates phosphorylated level of Akt. Akt denotes non-phosphorylated level of Akt to confirm that similar amounts of Akt were added to each lane

tion of Akt by BDNF was inhibited completely by LY294002 (Fig. 2B).

Requirement of TrkB receptors in the maximal activation of PI-3 kinase/Akt by NMDA

Since both NMDA and BDNF can activate the PI-3 kinase/Akt pathway, we determined the requirement of TrkB receptors in Akt activation by a maximal neuroprotective concentration of NMDA. Addition of NMDA (100 μM) to cultured neurons for 3h activates Akt; pretreatment with MK-801 completely blocked NMDA-mediated phosphorylation of Akt (Fig. 3A). This result suggests that NMDA receptors specifically activate Akt. Moreover, K252a, the TrkB receptor inhibitor, partially decreased NMDA-induced phosphorylation of Akt suggesting that maximal activation of the PI-3 kinase/Akt pathway by NMDA involves both NMDA and TrkB receptors (Fig. 3A). The activation of Akt by BDNF (100 ng/ml) was blocked by K252a in cultured neurons, however, MK-801 failed to affect BDNF-induced Akt activation (Fig. 3B). This result indicates that phosphorylation of Akt by BDNF is strictly mediated by TrkB receptors.

Discussions

Stroke, traumatic brain injury, and chronic neurodegenerative disorders are leading causes of death and disability in the United States. Glutamate, the endogenous excitatory neurotransmitter required for normal physiological excitation, is thought to play an essential role in the pathophysiological processes of these disorders (Olney et al., 1971; Wieloch et al., 1985; Choi, 1988). Through binding of ionotropic glutamate receptors (iGluRs), glutamate results in the influx of sodium and calcium ions through the channels gated by these receptors (Choi, 1988). Intracellular events such as immediate early genes and subsequent effector gene expression, following activation of receptors, appear to be responsible for apoptosis-related cell death. Elucidating the molecular mechanisms underlying neurotoxicity and neuroprotection can broaden our understanding of the pathological changes that occur in these disorders and may lead to the development of strategies to target critical pathway(s).

NMDA receptors, an ionotropic glutamate receptor subtype, appear to play a major role in glutamate-mediated excitotoxicity (Choi, 1988; Koh and Choi, 1988a). We have employed cultured cerebellar granule cells as a model for glutamate-mediated excitotoxicity and neuroprotection. Overactivation of NMDA receptors results in neuronal cell death in rat cerebellar granule cells (Favaron et al., 1988; Novelli et al., 1988; Schramm et al., 1990). Paradoxically, subtoxic concentrations of NMDA result in neuroprotection from glutamate toxicity (Marini and Novelli, 1991; Marini and Paul, 1992; Marini et al., 1998; Lipsky et al., 2001). Recent studies showed NMDA antagonists may increase neurodegeneration in mature rat brain undergoing slowly progressing neurodegeneration (Ikonomidou, 2000). In addition, NMDA receptors also mediate adaptive, plastic responses (long-term potentiation) (Ghosh and Greenberg, 1995; Katz and

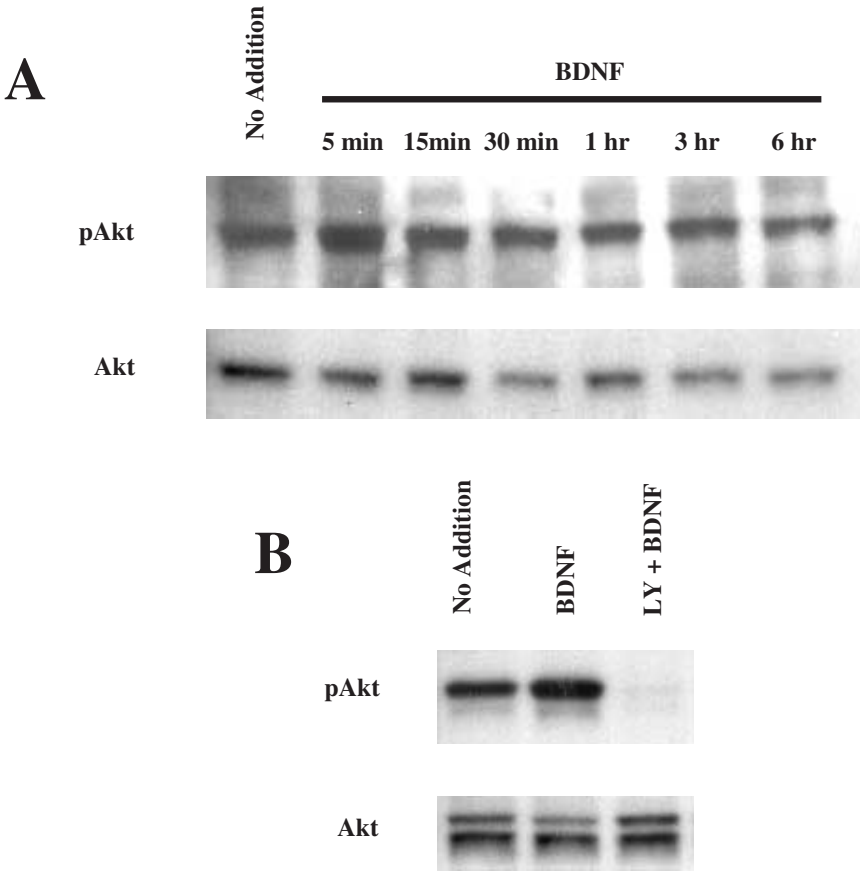

Fig. 2. Activation of PI-3 kinase/Akt by BDNF. **A** Cells were treated with medium alone (cont) or BDNF (100 ng/ml) for different periods (from 5 minutes to 6 hours), and then cell lysate were collected and subjected to Western blot analysis as described in Materials and Methods. **B** Cells were incubated with medium alone (cont), 100 ng/ml BDNF (BDNF), or 10 μM LY294002 plus BDNF (LY + BDNF) for 5 minutes. Cells were exposured to LY294002 for 1 hour prior to the addition of BDNF. pAkt indicates phosphorylated level of Akt and Akt indicates non-phosphorylated level of Akt

Shatz, 1996). The molecular mechanisms mediating such opposing responses are unclear.

We have been using cultured rat cerebellar granule cells to study the neuroprotective mechanisms of NMDA based on many features of this cell culture model. The neurons are relatively homogenous and express all of the glutamate receptor subtypes. The neurons are responsive to various neurotrophic factors and in particular to the neurotrophins (Lindholm et al., 1993; Segal et al., 1995), a family of trophic factors related by primary amino acid sequence homology, including BDNF, NGF, NT-3, and NT-4/5 (Levi-Montalcini, 1987; Hohn et al., 1990; Maisonpierre et al., 1990; Jones and Reichardt, 1990; Rosenthal et al., 1990). TrkA, TrkB, and TrkC, high affinity receptors having a similar intrinsic protein-tyrosine kinase activity but different ligand binding properties, are the receptors for NGF, BDNF, and NT-3, respectively (Rodriguez-Tebar et al., 1990; Lamballe et al, 1991; Klein et al., 1991).

We have shown that the subtoxic concentration of NMDA- mediated neuroprotection against glutamate toxicity can be inhibited by the RNA synthesis inhibitor actinomycin D or the protein synthesis inhibitor cycloheximide in cerebellar granule cells (Marini et al., 1992). Thus, NMDA receptor-mediated neuroprotection requires new RNA and protein synthesis and therefore appears to be mediated by the expression of neuroprotective protein(s). These data demonstrate the presence of an active NMDA receptor-mediated and transcriptionally directed neuroprotective mechanism in cerebellar granule cells. Our recent studies (Marini et al., 1998) demonstrated that subtoxic concentrations of NMDA evoked an accumulation of BDNF in the medium and time-dependent increase in BDNF mRNA. The increase of BDNF in the medium is followed by the enhanced TrkB phosphorylation, suggesting that NMDA increases the release of BDNF and therefore the activity of TrkB receptors. The data indicates that NMDA exerts its neuroprotection through activating the TrkB receptor via a BDNF autocrine loop. However, the intracellular signaling pathways involved in NMDA neuroprotection are still not clear and need to be characterized to enhance further understanding of the molecular mechanisms.

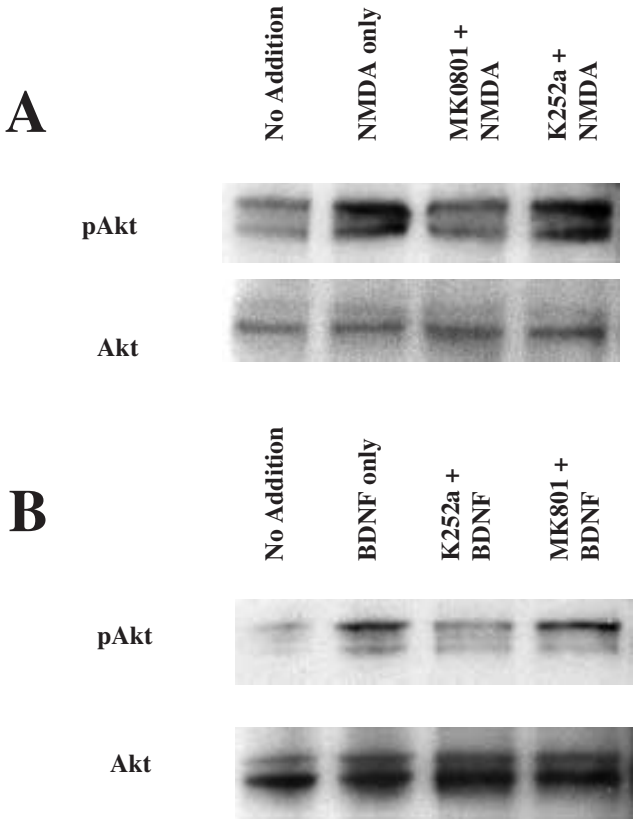

Fig. 3. Effect of MK-801 and K252a on NMDA and BDNF induced PI-3 kinase/Akt activation. **A** Cells were incubated with medium alone (cont), 100 μM NMDA (NMDA), MK-801 (1 μM) plus NMDA (MK-801 + NMDA) or K252a (10 nM) plus NMDA (K252a + NMDA) for 3 hours. **B** Cells were incubated with medium alone (cont), 100 ng/ml BDNF (BDNF), K252a (10 nM) plus BDNF (K252a + BDNF) or MK-801 (1 μM) plus BDNF (MK-801 + BDNF) for 5 minutes. MK801 or K252a was added to the medium 1 hour before the addition of NMDA. Cell lysate preparation and Western blot analysis were performed as described in Materials and methods. pAkt denotes phosphorylated level of Akt whereas Akt indicates non-phosphorylated level of Akt

There are two different kinds of signal transduction pathways in the neurons designated as the "positive" signal transduction cascades promoting survival, and the "negative" cascades promoting apoptosis (Williams et al., 1999). The balance between the two pathways determines the fate of a neuron. So, up-regulation of neuroprotective pathway and/or down-regulation of apoptotic pathway may occur in NMDA neuroprotective events. PI-3 kinase/Akt signal transduction cascade has been shown to be an important survival-promoting pathway in neurons and non-neuronal cells in vitro (Dudek et al., 1997; Blair et al., 1999; Kim et al., 2000; Ng et al., 2000; Zhang et al., 2000; Encinas et al., 2001). NGF-mediated activation of the PI-3 kinase/Akt pathway appears to be required for the survival effects in pheochromocytoma 12 (PC12) (Yao and Cooper, 1995). Activation of PI-3 kinase/Akt pathway by insulin-like growth factor-1 in cerebellar granule neurons is also important for survival (Dudek et al., 1997; Miller et al., 1997). We hypothesized that PI-3 kinase/Akt pathway may be important in NMDA neuroprotection and determined the effect of NMDA and BDNF on the activation of PI-3 kinase/Akt pathway as a first step in understanding cooperative events leading to a neuroprotective state.

NMDA increased the phosphorylation of Akt in a time-dependent manner with maximal activation at 3 hours. However, BDNF activated Akt significantly as early as 5 minutes. In our previous work (Marini et al., 1998), we showed an early (by 2–5 min) accumulation of BDNF in the medium of neurons incubated with NMDA. This suggests that activation of Akt is an early event by NMDA-induced release of BDNF. In fact, a small increase in Akt phosphorylation by NMDA within 5 min is observed, however, maximal Akt activation is a later event in comparison to BDNF. Activation of Akt by NMDA and BDNF is inhibited by the PI-3 kinase inhibitor, LY294002. These results indicate that Akt is a downstream target of PI-3 kinase and activation of Akt by NMDA and BDNF is through PI-3 kinase. The NMDA-mediated increase in Akt phosphorylation was blocked by the NMDA receptor antagonist MK-801, demonstrating that activation is through NMDA receptors. The TrkB receptor antagonist, K252a, decreased NMDA-mediated phosphorylation of Akt. This indicates that activated TrkB receptors are required for the maximal activation of Akt by NMDA consistent with our previous findings that activation of TrkB receptor via a BDNF autocrine loop underlies the neuroprotective effect of NMDA. K252a but not MK-801 blocked BDNF-induced activation of Akt, suggesting that BDNF exerts its intracellular event through TrkB receptors, not NMDA receptors. Taken together, PI-3 kinase/Akt pathway is the co-intracellular target of both NMDA and BDNF. Activation of PI-3 kinase/Akt signaling by NMDA involves NMDA receptors as well as TrkB receptors. The time difference of NMDA and BDNF on the activation of PI-3 kinase/Akt suggests their different roles in eliciting intracellular time-related events in the neurons.

Previous studies showed that PI-3 kinase/Akt signaling promotes endothelial cell survival by inhibit-

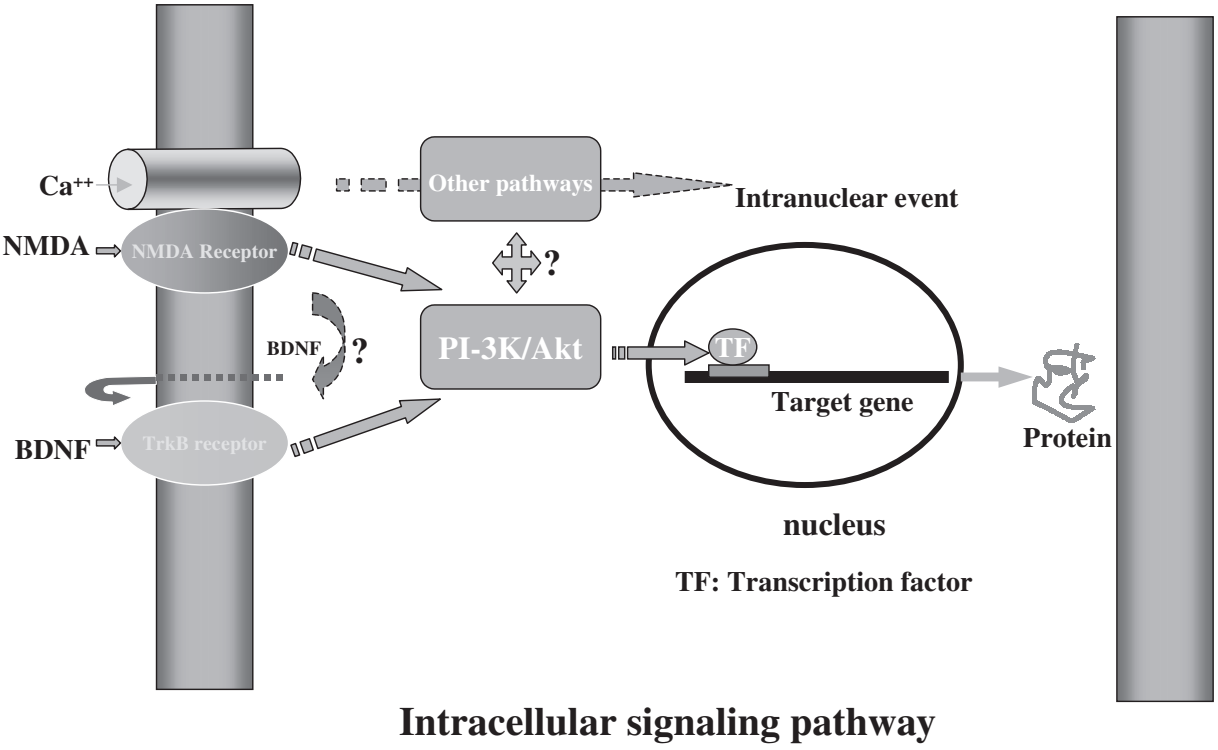

Fig. 4. Schematic representation of activity-dependent activation of Akt by NMDA and TrkB receptors. Activation of NMDA receptors results in the release of BDNF which in turn activates its cognate receptor TrkB. TrkB activation rapidly activates the PI-3 kinase/Akt pathway but co-activation of NMDA and TrkB receptors is required for maximal Akt activation. Whether activated NMDA and TrkB receptors interact is unknown. Also unknown is whether there is cross-talk between the PI-3 kinase/Akt pathway and other signal transduction pathways leading to transcription factor-mediated gene regulation

ing p38 MAP kinase dependent apoptosis in vascular endothelial cells (Gratton et al., 2001). INFα/β has also been demonstrated to promote cell survival by activating NF-κB through PI-3 kinase and Akt in human Daudi cells (Yang et al., 2001). Our recent data demonstrated that NF-κB is a critical determinant in NMDA neuroprotection (Lipsky et al., 2001).

Based on the findings in this study, we propose that activity-dependent release of BDNF by NMDA rapidly activates Akt first through TrkB receptors followed by NMDA receptors. We suggest further that the activated PI-3 kinase/Akt pathway is sustained by NMDA and TrkB receptors. Activation of TrkB and NMDA receptors rapidly and maximally activate the PI-3 kinase/Akt pathway that may lead to gene transcription and induce a neuroprotective state (Fig. 4). Therefore, the PI-3 kinase/Akt pathway may be one of the signal transduction pathways responsible for NMDA-mediated neuroprotection in granule cell neurons. Whether activated NMDA and TrkB receptors interact is unknown. Also unknown is whether there are other signal transduction pathways that converge to regulate gene transcription to induce a neuroprotective state (Fig. 4).

Future experiments will examine the roles of other signal transduction pathways and some important transcription factors targeted by PI-3 kinase/Akt in NMDA neuroprotection. It will also be important to investigate possible cross-talk between signaling pathways resulting in a synergism or potentiation of effects. Once these pathways have been elucidated and the mechanism(s) delineated, novel strategies can be employed to prevent neuronal cell death and promote survival.

References

Blair LA, Bence-Hanulec KK, Mehta S, Franke T, Kaplan D, Marshall J (1999) Akt-dependent potentiation of L channels by insulin-like growth factor-1 is required for neuronal survival. J Neurosci 19: 1940–1951

Choi DW (1988) Glutamate neurotoxicity and disease of the nervous system. Neuron 1: 623–634

Choi DW (1995) Calcium: still center-stage in hypoxic-ischemic neuronal death. Trends Neurosci 18: 58–60

Datta K, Bellacosa A, Chan TO, Tsichlis PN (1996) Akt is a direct target of the phosphatidylinositol 3-kinase. J Biol Chem 271: 30835–30839

Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME (1997) Regulation of neuronal survival by the serine-threonine protein kinase Akt. Science 275: 661–664

Encinas M, Tansey MG, Tsui-Pierchala BA, Comella JX, Milbrandt J, Johnson EM Jr (2001) C-src is required for glial cell line-derived neurotrophic factor (GDNF) family ligand-mediated neuronal survival via a phosphatidylinositol-3 kinase (PI-3K)-dependent pathway. J Neurosci 21: 1464–1472

Favaron M, Manev H, Alho H (1988) Gangliosides prevent glutamate and kainate neurotoxicity in primary neuronal cultures of neonatal rat cerebellum and cortex. Proc Natl Acad Sci USA 85: 7351–7355

Franke TF, Kaplan DR, Cantley LC, Toker A (1997) Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-biphosphate. Science 275: 665–668

Ghosh A, Greenberg ME (1995) Calcium signaling in neurons: molecular mechanisms and cellular consequences. Science 268: 239–247

Gratton JP, Morales-Ruiz M, Kureishi Y, Fulton D, Walsh K, Sessa WC (2001) Akt down regulation of p38 signaling provides a novel mechanism of VEGF mediated cytoprotection in endothelial cells. J Biol Chem (in press)

Hohn A, Leibrock J, Bailey K, Barde Y-A (1990) Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. Nature 344: 339–341

Ikonomidou C, Stefovska V, Turski L (2000) Neuronal death enhanced by N-methyl-D-aspartate antagonists. Proc Natl Acad Sci USA 97: 12885–12890

Jones KR, Reichardt LF (1990) Molecular cloning of a human gene that is a member of the nerve growth factor family. Proc Natl Acad Sci USA 87: 8060–8064

Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274: 1133

Kim I, Kim JH, Moon SO, Kwak HJ, Kim NG, Koh GY (2000) Angiopoietin-2 at high concentration can enhance endothelial cell survival through the phosphatidylinositol 3-kinase/Akt signal transduction pathway. Oncogene 19: 4549–4552

Klein R, Jing S, Nanduri V, O'Rourke E, Barbacid M (1991) The trk proto-oncogene encodes a receptor for nerve growth factor. Cell 65: 189–197

Koh J, Choi DW (1988a) Vulnerability of cultured cortical neurons to damage by excitotoxins: differential susceptibility of neurons containing NADPH-diaphorase. J Neurosci 8: 2153–2163

Koh J, Choi DW (1988b) Zinc alters excitatory amino acid neurotoxicity on cortical neurons. J Neurosci 8: 2164–2171.

Lamballe F, Klein R, Barbacid M (1991) TrkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. Cell 66: 967–979

Levi-Montalcini R (1987) The nerve growth factor 35 years later. Science 237: 1154–1162

Lindholm D, Dechant G, Heisenberg CP, Thoenen H (1993) Brain-derived neurotrophic factor is a survival factor for cultured rat cerebellar granule neurons and protects them against glutamate-induced neurotoxicity. Eur J Neurosci 5: 1455–1464

Lipsky RH, Xu K, Zhu D, Kelly C, Terhakopian A, Novelli A, Marini AM (2001) Nuclear factor kappa B is a critical determinant in N-methyl-D-aspartate receptor-mediated neuroprotection. J Neurochem 78: 254–264

Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME, Lindsay R, Yancopoulos GD (1990) Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. Science 247: 1446–1451

Marini AM, Novelli A (1991) The glutamate uptake blocker DL-threo-3-hydroxyaspartate reduces NMDA receptor activation by glutamate in cultured neurons. Europ J Pharm 194: 131–132

Marini AM, Paul SM (1992) N-methyl-D-aspartate receptor-mediated neuroprotection in cerebellar granule cells requires new RNA and protein synthesis. Proc Natl Acad Sci USA 89: 6555–6559

Marini AM, Rabin SJ, Lipsky RH, Mocchetti I (1998) Activity-dependent release of brain-derived neurotropic factor underlies the neuroprotective effect of N-methyl-D-aspartate. J Biol Chem 273:29394–29399

Miller TM, Tansey MG, Johnson EM Jr, Creedon DJ (1997) Inhibition of phosphatidylinositol 3-kinase activity blocks depolarization- and insulin-like growth factor I- mediated survival of cerebellar granule cells. J Biol Chem 272: 9847–9853

Ng SSW, Tsao MS, Chow S, Hedley DW (2000) Inhibition of phosphatidylinositol 3-kinase enhances gemcitabine-induced apoptosis in human pancreatic cells. Cancer Res 60: 5451–5455

Novelli A, Reilly JA, Lysko PG, Henneberry RC (1988) Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res 451: 205–212

Olney JW, Ho OL, Rhee V (1971) Cytotoxic effects of acidic and sulphur-containing amino acids on the infant mouse central nervous system. Exp Brain Res 14: 61–76

Rodriguez-Tebar A, Dechant G, Barde YA (1990) Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. Neuron 4: 487–492

Rosenthal A, Goeddel DV, Nguyen T, Lewis M, Shih A, Laramee GR, Nikolics K, Winslow JW (1990) Primary structure and biological activity of a novel human neurotrophic factor. Neuron 4: 767–773

Schramm M, Eimerl S, Costa E (1990) Serum and depolarizing agents cause acute neurotoxicity in cultured cerebellar granule cells: role of the glutamate receptor responsive to N-methyl-D-aspartate. Proc Natl Acad Sci USA 87: 1193–1197

Segal RA, Pomeroy SL, Stiles CD (1995) Axonal growth and fasciculation linked to differential expression of BDNF and NT3 receptors in developing cerebellar granule cells. J Neurosci 15: 4970–4981

Wieloch T (1985) Hypoglycemia-induced neuronal damage prevented by an N-methyl-D-aspartate antagonist. Science 230: 681–683

Williams EJ, Doherty P (1999) Evidence for and against a pivotal role of PI-3 kinase in a neuronal cell survival pathway. Mol Cell Neurosci 13: 272–280

Yang CH, Murti A, Pfeffer SR, Kim JG, Donner DB, Pfeffer LM (2001) INFα/β promotes cell survival by activating NF-κB through phosphatidylinositol-3 kinase and Akt. J Biol Chem 276: 13756–13761

Yao R, Cooper GM (1995) Requirement for phosphatidylinositol 3-kinase in the prevention of apoptosis by nerve growth factor. Science 267: 2003–2006

Zhang L, Himi T, Morita I, Murota S (2000) Hepatocyte growth factor protects cultured rat cerebellar granule neurons from apoptosis via the phosphatidylinotositol-3 kinase/Akt pathway. J Neurosci Res 59: 489–496

Authors' address: Ann M. Marini, Department of Neurology Uniformed Services University of the Health Sciences, Bethesda, MD 20814, U.S.A., E-mail: Ann.Marini@NA.AMEDD.ARMY.MIL, Fax: 202-782-2295