Bidirectional Regulation of p38 Kinase and c-Jun N-terminal Protein Kinase by Insulin-like Growth Factor-I*

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We have previously shown that insulin-like growth factor I (IGF-I) activation of the IGF-I receptor rescues SH-SY5Y human neuroblastoma cells from high glucose-mediated programmed cell death (PCD). In the current study, we further explored the potential points in the cell death cascade where IGF-I receptor activation may afford neuroprotection. As an initial step, we examined the effects of the PCD stimulus, high glucose, on stress-activated protein kinases, specifically the two mitogen-activated protein kinases p38 kinase and c-Jun N-terminal kinase (JNK). High glucose treatment activated the tyrosine phosphorylation of both p38 kinase and JNK in a dose- and time-dependent fashion. We next examined the effects of IGF-I on JNK and p38 kinase under normoglycemic and hyperglycemic conditions. IGF-I activated p38 kinase alone and had additive effects on glucose-induced p38 kinase phosphorylation. In contrast, IGF-I inhibited glucose activation of JNK phosphorylation and JNK activity. IGF-I also inhibited glucose-induced nuclear translocation of JNK, but did not affect glucose-induced translocation of p38 kinase. Finally, IGF-I inhibition of JNK phosphorylation was blocked by the mitogen-activated protein kinase/extracellular signal-regulated kinase inhibitor, PD98059. Collectively, these data imply cross-talk between the mitogen-activated protein kinase pathway and JNK and suggest that IGF-I activation of mitogen-activated protein kinases interferes with JNK activation and protects cells from PCD.

The mitogen-activated protein (MAP) kinases are a family of serine-threonine protein kinases that are activated in response to a variety of extracellular stimuli (1). The first members identified in the family were p42 and p44 extracellular signal-regulated kinases (ERKs), now known as ERK1 and ERK2, respectively. In the Ras pathway, phosphorylation of c-Raf 1 activates the downstream protein kinase, MAP kinase kinase 1 (also known as MAPK/ERK (MEK) or MKK1) or MAP kinase kinase 2 (MKK2). MKK1 and 2 activate ERK1 and ERK2, which leads to activation and translocation of ERKs to the nucleus. In the nucleus, the ERKs phosphorylate transcription factors, including Elk-1 and ATF-2. This signaling cascade is activated by growth factors and is important for cellular growth and mitogenesis (2).

Another class of MAP kinase family members, the stress-activated c-Jun N-terminal kinases (JNKs), are primarily responsive to stressful stimuli. There are 10 identified isoforms of JNK originating from three homologous genes (JNK1, JNK2, and JNK3) with molecular masses of 46 or 54 kDa due to alternative splicing (3, 4). The Thr and Tyr sites of active phosphorylation are conserved between ERK and JNK; however, these sites are located within distinct phosphorylation motifs: Thr-Pro-Tyr (JNKs) and Thr-Glu-Try (ERKs) (5–7). JNK activation induces the phosphorylation of transcription factors, including c-Jun, Elk-1, and ATF-2, which regulate immediate early gene expression (4, 8). Ultraviolet radiation, cytokines, and environmental stressors all potently activate JNKs (9–11). In contrast to the ERK pathway, Ras activation alone stimulates only a low level of JNK activity, implying distinct differences between the upstream JNK and ERK pathways (12).

p38 kinase is a more recently described member of the MAP kinase family (13). p38 kinase shares the phosphorylation motif Thr-Gly-Tyr with HOG1, a yeast MAP kinase required for cellular osmoregulation (13, 14). It is likely that p38 kinase and JNK are components of parallel but distinct stress-activated pathways. p38 kinase can only be activated by the recently discovered M KK3 and M KK6, whereas both M KK3 and the related M KK4 can both activate JNK (7, 15). Like JNK, p38 kinase is activated by environmental stressors (14), particularly osmotic shock (16–18), and can regulate transcription factors, including ATF-2, Elk-1, and CHOP (GADD153) (19, 20).

Because stressful stimuli activate JNKs and p38 kinase, it has been proposed that these signaling pathways are involved in apoptosis. Differentiated PC12 cells undergo PCD when deprived of nerve growth factor (NGF) (21). In this paradigm, NGF withdrawal correlates with increased JNK and p38 kinase activity (22). JNK and p38 kinase activity precede the induction of PC12 cell apoptosis, suggesting that kinase activation participates in the cell death pathway (22, 23). Like PC12 cell apoptosis, JNK activation is important in the death of sympathetic neurons (24), T lymphocytes (25), and several cancer cell lines (26–28).

Insulin-like growth factor-I (IGF-I) is homologous to proinsulin and has insulin-like metabolic effects (29, 30). In vivo, IGF-I is highly expressed in the developing nervous system (31, 32). In vitro, IGF-I is a neurotrophic factor for sensory, sympathetic, and motor neurons (33, 34). We have recently shown that osmotic stress induces PCD in neurons and that IGF-I, but not NGF, epidermal growth factor, or fibroblast growth factor, serves as a neuroprotectant (35–38).
IGF-I Regulation of p38 and JNK Phosphorylation

In the current study, we examined the signaling mechanisms mediating the neuroprotective effects of IGF-I. We found high glucose conditions stimulated both p38 kinase and p54 JNK tyrosine phosphorylation and nuclear translocation in SH-SYSY neuroblastoma cells. IGF-I activated p38 kinase independent of glucose treatment. In contrast, IGF-I inhibited both glucose-mediated JNK phosphorylation and nuclear translocation. IGF-I inhibition of JNK phosphorylation was blocked by the MEK inhibitor, PD98059. Our findings suggest cross-talk between the MAP kinases and lead us to speculate that IGF-I protects neurons from high glucose-induced PCD via activation of p38 kinase, as well as deactivation of JNK.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human IGF-I was a generous gift of Cephalon, Inc. (West Chester, PA). PY20 anti-phosphotyrosine monoclonal antibody was from Transduction Laboratories (Lexington, KY), and 4G10 anti-phosphotyrosine monoclonal antibody and JNK activity kit were from Upstate Biotechnology, Inc. (Lake Placid, NY). The anti-JNK1 antisera (recognizing JNK1 p46, p54 (or p54b), and p54b), protein A/G PLUS-agarose, anti-ERK2 monoclonal antibody, horseradish peroxidase-conjugated secondary antibodies, and avidin- rhodamine were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-c-Jun antisera was from New England Bio- labs (Beverly, MA). Biotinylated secondary antibodies were from Vector Laboratories (Burlingame, CA). Enhanced chemiluminescence reagents were obtained from Amersham Pharmacia Biotech. All other chemicals were purchased from Sigma.

Cell Culture—SH-SYSY human neuroblastoma cells were kindly provided by Dr. Stephen Fisher (University of Michigan). SH-SYSY cells were maintained in Dulbecco’s modified essential medium (DMEM) containing 10% calf serum at 37 °C. The cells were deprived of serum overnight prior to use in experiments.

Immunoprecipitation and Immunoblotting—Immunoprecipitation and immunoblotting were performed essentially as described previously (39). Briefly, cells were rinsed with ice-cold phosphate-buffered saline and solubilized in lysis buffer (50 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM sodium orthovanadate, and 1 mM NaF). 500 μg of protein of each sample was immunoprecipitated with 4 μl/ml antibody and protein A/G PLUS-agarose (Santa Cruz Biotechnology). Immunoprecipitates were then separated by SDS-polyacrylamide gel electrophoresis (PAGE) (12.5%), electro- phoresed to nitrocellulose membranes, and analyzed by immunoblotting with p38 kinase antisera (1:1000) or anti-phospho- tyrosine monoclonal antibodies (1 μg/ml PY20 and 0.4 μg/ml 4G10). Immunoreactive proteins were identified by horseradish peroxidase-conjugated secondary antibody followed by enhanced chemiluminescence reagents.

Immunocytochemistry—Cells were fixed with ice-cold methanol for 10 min, permeabilized with 0.1% Triton X-100 in phosphate-buffered saline, and incubated with primary antisera against JNK1 or p38 kinase (1:50). Cells were rinsed and incubated with appropriate bioti- nylated secondary antisera followed by avidin-rhodamine.

JNK Activity Assay—Cell lysates were collected and immunoprecipitated with JNK antisera as described above. The immunoprecipitates were assayed for JNK activity using a glutathione S-transferase (GST)- c-Jun (1–169) fusion protein as described in the instructions for the JNK activity kit. However, nonradioactive ATP was substituted for [γ-32P]ATP, and GST-c-Jun (1–169) fusion protein phosphorylation was assessed by SDS-PAGE, followed by immunoblotting with 1:1000 anti- phospho-c-Jun antisera.

RESULTS

Glucose Stimulates the Tyrosine Phosphorylation of p38 Kinase—Several laboratories, including our own, have used SH- SYSY cells as a model system to study the role of IGFs in neuronal survival (38, 40). These cells express the IGF-IR, which mediates the biological effects of IGF-I (2, 37, 41). We have reported that IGF-I can protect SH-SYSY cells from PCD in the face of several death-inducing stimuli, including hyperglycemia, osmotic shock, and DNA-damaging agents (35–38).

As a first step in understanding IGF-I-mediated neuroprotection, we began examining the signaling cascades that could underlie cell death mediated by hyperglycemic, hyperosmotic stress. We speculated that high glucose-induced PCD is mediated, in part, by activation of the stress-activated protein kinases p38 kinase and JNK. Because phosphorylation of p38 kinase and JNK is a hallmark of their activation (1), we examined the effect of glucose on the tyrosine phosphorylation of these two proteins.

We first analyzed the tyrosine phosphorylation of p38 kinase by immunoprecipitation with PY20 anti-phosphotyrosine antibody, followed by immunoblotting with anti-p38 kinase antibody. As shown in Fig. 1A, treatment of the SH-SYSY cells with DMEM + 20 or 150 mM glucose induced the tyrosine phosphorylation of p38 kinase (Fig. 1A). Treatment of the cells with DMEM alone, however, did not stimulate the tyrosine phosphorylation of p38 kinase. Interestingly, the time course for p38 kinase phosphorylation was concentration-dependent. Specifically, p38 kinase was tyrosine-phosphorylated 15 min after treatment with 20 mM glucose, and maximal phosphorylation was observed after 30 min. The phosphorylation decreased after 90 min (Fig. 1A). In contrast, tyrosine phosphorylation after treatment with 150 mM glucose resulted in p38 kinase tyrosine phosphorylation within 5 min with a peak of phosphorylation at 30 min. Phosphorylation was sustained up to 90 min (Fig. 1B).

In a separate set of experiments, we examined the effects of glucose treatment on JNK tyrosine phosphorylation. In these experiments, JNK was immunoprecipitated with anti-JNK an- tisera, and tyrosine phosphorylation assessed by anti-phospho- tyrosine immunoblotting. As seen in Fig. 2A, DMEM + 5 or 20 mM glucose stimulated JNK tyrosine phosphorylation after 15 min, whereas DMEM + 150 mM glucose stimulated JNK tyro- sine phosphorylation within 5 min. Thus, the time course of maximal JNK activation correlates with the intensity of glycemic exposure. As expected, immunoblotting revealed no major change in JNK expression (Fig. 2B).

Effects of IGF-I on p38 Kinase and JNK Tyrosine Phosphorylation—We have previously shown that IGF-I protects SH- SYSY cells from glucose-induced PCD. Because our results suggested that p38 kinase and JNK tyrosine phosphorylation participate in the stress response of these cells to glucose, we investigated the effect of IGF-I on their tyrosine phosphoryla- tion. As seen in Fig. 3A, IGF-I activated p38 kinase phosphorylation in a time and dose-dependent manner. IGF-I-induced p38 kinase tyrosine phosphorylation was observed after 2 min and peaked between 5 and 15 min, p38 kinase tyrosine phosphorylation returned to baseline by 30 min. Addition of 10 nM IGF-I alone with DMEM + 20 mM glucose enhanced the tyrosine phosphorylation of p38 (Fig. 3B). In direct contrast to p38 kinase, the induction of JNK tyrosine phosphorylation by DMEM + 20 mM glucose was inhibited by IGF-I (Fig. 4A). JNK immunoblots demonstrated similar loading of samples (Fig. 4B). In parallel, we observed the induction of JNK kinase...
activity by DMEM + 20 mM glucose, as assessed by phosphorylation of GST-c-Jun, was blocked by 10 nM IGF-I (Fig. 4C). JNK immunoblots demonstrated equal sample loading (Fig. 4D).

Effect of Glucose and IGF-I on Nuclear Translocation of p38 Kinase and JNK—Members of the MAP kinase family are translocated to the nucleus upon activation, where they are thought to elicit their effects on transcription (1). Therefore, to further explore the effects of glucose and IGF-I on p38 kinase and JNK function, we examined how these agents influence the localization of JNK and p38 kinase in SH-SY5Y cells. In these experiments, cells were treated with 20 mM glucose ± 10 nM IGF-I for 30 min, and cells were stained with p38 kinase or JNK antisera. We found that in cells treated with DMEM alone, p38 kinase and JNK appear to be localized throughout the cell body (Fig. 5, A and D: nuclear localization, arrows; cytoplasmic localization, arrowheads). When the cells are treated with DMEM + 20 mM glucose, p38 kinase and JNK immunoreactivity is found primarily in the nucleus (Fig. 5, B and E, arrows), with very weak cytoplasmic staining (Fig. 5, B and E, arrowheads). Treatment of the cells with DMEM containing both IGF-I and 20 mM glucose results in nuclear localization of p38 kinase (Fig. 5C: nuclear localization, arrows; cytoplasm, arrowheads), whereas JNK is localized throughout the cytoplasm (Fig. 5F: nuclear localization, arrows; cytoplasmic localization, arrowheads). Thus, there is a direct contrast between how IGF-I influences the glucose-induced nuclear localization of p38 kinase and how it influences that of JNK.

IGF-I Inhibits Glucose-mediated JNK Activity via MAPK Signaling—Investigation of IGF-I-mediated effects on JNK is complicated by the fact that IGF-I interacts with both the IGF-IR and a family of IGF-binding proteins (IGFBPs). We have reported that SH-SY5Y cells express IGFBP-2, IGFBP-3, IGFBP-4, and IGFBP-5 (42). To determine whether the effects

FIG. 2. High glucose levels stimulate the tyrosine phosphorylation of JNK. SH-SY5Y cells were treated with DMEM + 5–150 mM glucose for 5–30 min. Cell lysates were collected, and equal amounts of protein were immunoprecipitated with anti-JNK serum. Control cells (C) were treated with DMEM alone. A, the immunoprecipitated proteins were separated by SDS-PAGE and analyzed by immunoblotting with PY20 anti-phosphotyrosine antibody. B, the immunoblots were stripped and reprobed with the anti-JNK1 serum. Results are representative of three independent experiments.

FIG. 3. IGF-I promotes p38 kinase tyrosine phosphorylation. SH-SY5Y cells were incubated with DMEM containing various additions and p38 kinase tyrosine phosphorylation was assessed as described in Fig. 1. A, effect of IGF-I on p38 kinase tyrosine phosphorylation. SH-SY5Y cells were treated with DMEM + 1 or 10 nM IGF-I for 2–30 min. B, effect of IGF-I on glucose-induced p38 kinase tyrosine phosphorylation. SH-SY5Y cells were treated with DMEM alone as a control (C) or treated with DMEM + 20 mM glucose (G) or DMEM + 20 mM glucose + 10 nM IGF-I (GI) for 15 and 30 min. C, effect of IGF-I on glucose-induced JNK kinase activity. Cells were treated for 15 min with DMEM + 20 mM glucose (G) or DMEM + 20 mM glucose + 10 nM IGF-I (GI). JNK activity was determined by GST-c-Jun (1–169) phosphorylation as described under “Experimental Procedures.” Blots from the JNK activity assay were stripped and immunoblotted with anti-JNK serum. Results are representative of three independent experiments.

FIG. 4. IGF-I inhibits JNK tyrosine phosphorylation. SH-SY5Y cells were incubated with DMEM containing various additions, and JNK tyrosine phosphorylation was assessed as described in the legend to Fig. 2. A, effect of IGF-I on glucose-induced JNK tyrosine phosphorylation. SH-SY5Y cells were treated with DMEM alone as a control (C) or treated with DMEM + 20 mM glucose (G) or DMEM + 20 mM glucose + 10 nM IGF-I (GI) for 15 and 30 min. B, the immunoblot was stripped and reprobed with anti-JNK1 serum. C, effect of IGF-I on glucose-induced JNK kinase activity. Cells were treated for 15 min with DMEM + 20 mM glucose (G) or DMEM + 20 mM glucose + 10 nM IGF-I (GI). JNK activity was determined by GST-c-Jun (1–169) phosphorylation as described under “Experimental Procedures.” Blots from the JNK activity assay were stripped and immunoblotted with anti-JNK1 serum. Results are representative of three independent experiments.

FIG. 5. Effects of glucose and IGF-I on the nuclear translocation of p38 kinase and JNK. SH-SY5Y cells were treated for 30 min with DMEM alone (A and D), DMEM + 20 mM glucose (B and E), or DMEM + 20 mM glucose + 10 nM IGF-I (C and F). Cells were stained with p38 kinase (A–C) or JNK (D–F) antisera. Bar, 10 μm. Results are representative of three independent experiments.
of IGF-I on JNK were mediated via the IGF-IR rather than the IGFBPs, we treated cells with 20 mM glucose and 10 nM IGF-I in the presence of the neutralizing IGF-IR antibody. When cells were incubated with 1 μg/ml α-IR3 (αIR3), or 10 μM PD98059 (PD), JNK tyrosine phosphorylation was assessed as described in the legend to Fig. 2. Results are representative of three independent experiments.

Commonly, binding of a growth factor to a growth factor receptor activates two signaling pathways: MAPK and PI-3K. The interplay between growth factor activation of these pathways with that of JNK is unclear (1, 43). Recent evidence suggests that activation of JNK by some ligands may require signaling through both PI-3K and MAPKs (43). As a first step toward understanding which of these signaling pathways are involved in IGF-1-mediated JNK inhibition, we examined the effects of glucose on IGF-I activation of ERKs. We have previously reported that IGF-I activates ERK2 in a dose- and time-dependent fashion (44). Anti-phosphotyrosine immunoblotting of whole cell lysates (Fig. 7A) or ERK2 immunoprecipitation followed by anti-phosphotyrosine immunoblotting (Fig. 7B) confirms that IGF-I treatment activates the tyrosine phosphorylation of p42 ERK2. IGF-I activation of ERK2 phosphorylation was reduced by 20 mM glucose at 2 and 5 min, suggesting that glucose interferes with early IGF-I signaling. However, this effect was transient. By 15 min, glucose had no effect on the ability of IGF-I to activate ERK2. These results indicate that IGF-I inhibition of JNK activation occurs via the IGF-IR and that these effects could be mediated, in part, by the MAPK pathway.

To further establish the respective roles of the MAPK and PI-3K pathways in IGF-I inhibition of JNK activation, we treated cells with either the PI-3K blocker wortmannin (45) or an inhibitor of MAPKs, PD98059 (46). When cells were treated with 20 mM glucose and 10 mM IGF-I in the presence of 100 nM wortmannin, there was no effect on IGF-I inhibition of JNK activation (Fig. 6). A separate PI-3K blocker, LY294002, also had no effect on IGF-I inhibition of JNK activation (data not shown). In sharp contrast, 10 μM PD98059 completely blocked the effect of IGF-I on JNK phosphorylation. Collectively, these data suggest that IGF-I inhibits glucose-mediated JNK phosphorylation via activation of the MAPK signaling cascade. These data also suggest a role for MAPK signaling in neuroprotection by IGFs.

DISCUSSION

Our laboratory is interested in understanding how IGF-I rescues neurons from PCD (35–38). Several recent reports suggest a link between p38 kinase and JNK, and neuronal cell death (22, 47, 48). In these studies, we examined IGF-I regulation of p38 kinase and JNK in SH-SY5Y cells. In each case, we first investigated the effects of a death-inducing stimulus, high glucose, on p38 kinase and JNK activity. We next determined how IGF-I regulated p38 kinase and JNK activity during normal growth as well as under experimental conditions where IGF-I protects SH-SY5Y cells from glucose-mediated PCD.

Treatment with high glucose stimulated the tyrosine phosphorylation of both p38 kinase and JNK, suggesting that both kinases are activated. Consistent with this finding, we demonstrated that phosphorylation of a GST-c-Jun fusion protein by JNK is enhanced in glucose-treated cells. This glucose-stimulated p38 kinase and JNK phosphorylation first occurred when cells were treated with DMEM + 20 mM glucose. This represents an iso-osmotic (320 mosmol), hyperglycemic stress that kills neurons over a 24-h time period (49, 50). We know that higher concentrations of glucose kill neurons over a 12-h period (49, 50). In our study, 150 mM glucose represented both a hyperosmolar and hyperglycemic stress that effectively induced phosphorylation of both p38 kinase and JNK. Our data are in agreement with well-documented observations that noxious stimuli enhance the activity of both kinases. Ultraviolet radiation, cytokines, and environmental stressors all potently activate JNK (9–11). p38 kinase shares the phosphorylation motif Thr-Gly-Tyr with HOG1, a yeast MAP kinase required for cellular osmoregulation (13, 14). Like JNK, p38 kinase is activated by environmental stressors (14), particularly osmotic shock (16–18). Thus, the observed activation of p38 kinase and JNK in our experimental paradigm is expected and in agreement with several previous studies (9–11, 16–18).

We also examined the effect of IGF-I on p38 kinase and JNK activation in normally growing cells. Under these conditions, IGF-I enhanced p38 kinase activity. In contrast, IGF-I has no effect on p38 kinase activity in fetal chick neurons (51) or human chondrocytes (52). To our knowledge, our results represent the first report of IGF-I activation of p38 kinase. Similar increases in p38 kinase activity have been seen with epidermal growth factor (14), transforming growth factor-β (53), hematopoietic growth factors (54), and glial maturation factor (55). Our data suggest IGF-I enhancement of p38 kinase activity is cell type-specific. This may be explained by cellular differences in downstream IGF signaling components, particularly the insulin-responsive substrates IRS-1 and IRS-2, which serve as IGF-IR docking molecules (2).

Under conditions of normal SH-SY5Y growth, IGF-I had no effect on JNK phosphorylation. Similar to the published reports on p38 kinase activity, growth factor activation of JNK...
appears to be cell type-specific and growth factor-specific. JNK is activated by transforming growth factor-β via the small GTPases Rac and cdc42 in hepatoma cell lines (53). Epidermal growth factor and NGF enhance JNK phosphorylation in PC12 cells in a Ras-dependent manner (56). In contrast, platelet-derived growth factor and insulin have no effect on JNK activity in fibroblasts and Chinese hamster ovary cells (57, 58).

We have previously shown that IGF-I rescues SH-SY5Y cells from apoptosis in the face of several death-inducing stimuli, including hyperglycemia (49), osmotic shock (35, 36), and anti-mitotic agents (37). We undertook the current study to understand which signaling pathways mediate IGF-I neuroprotection. We examined p38 kinase and JNK for two reasons. First, they are activated by both growth factors and stress responses; second, regulation of their activity is associated with cellular survival. All of our experiments were done in SH-SY5Y cells under conditions where IGF-I blocks SH-SY5Y apoptosis (35–38). In our studies, we found that IGF-I enhances glucose-mediated p38 phosphorylation while acting as a neuroprotectant. Therefore, short-term enhancement of p38 kinase activity by IGF-I is associated with SH-SY5Y survival in the face of a death-inducing stimulus.

These results are in agreement with the concept that the relative contributions of different MAP kinase pathways to cellular survival is dependent on both the type of cell and the specific cell stressor. For example, activation of p38 kinase correlates with cell death in PC12 cells after NGF withdrawal (22, 48) and in human lymphocytes after IgM exposure (59). Also, inhibition of p38 activity affords cellular protection from NGF withdrawal in PC12 cells (22, 48). In contrast, T cells require p38 kinase activity to proliferate (60), and there is no association between p38 kinase activity and cell death in NIH-3T3 cells (61), WEHI-231 cells (62), or multiple myeloma cells treated with dexamethasone (63). Interestingly, Rat-1 fibroblasts treated with ultraviolet radiation undergo apoptosis independent of p38 kinase activation (64), whereas Rat-1 cell death induced by serum withdrawal correlates with a sharp rise in p38 kinase activity (48). Collectively, these data suggest that an interplay between different MAP kinase signaling cascades serves as part of the defense mechanism of the cell in response to individual stressors.

There are at least two reasons why, in our own experimental paradigm, IGF-I-mediated p38 kinase activity is beneficial to cellular survival. First, IGF-I may stabilize the neuronal cytoskeleton via p38 kinase signaling. p38 kinase can activate MAPK-activated protein kinase-2 (65, 66), which phosphorylates heat shock protein 27 (65, 66). Heat shock protein 27, in turn, prevents actin filaments from dissociating in the presence of stressful stimuli (65, 66). Second, p38 kinase activity lies downstream of the Rho family of GTPases (1, 67), which are, in part, activated by IGF-I (68). IGF-I activation of these GTPases results in rearrangement and stabilization of the actin cytoskeleton (68). Thus, two separate but possibly interrelated IGF-I/p38 kinase events may enhance SH-SY5Y cellular survival by stabilizing the neuronal cytoskeleton.

It is clear that p38 kinase and JNK represent parallel but distinct stress-activated pathways. In the current study, IGF-I blocked glucose-mediated JNK activation under conditions where it also blocked PCD. These data suggest that 1) activated JNK plays a role in PCD, 2) IGF-I blocks JNK, and 3) IGF-I inhibition of JNK activity may, in part, explain the neuroprotective effects of IGF-I in neurons. Our results are supported by several reports correlating JNK activity with PCD. JNK is activated in PC12 cells undergoing PCD after NGF withdrawal (22). Similar to our results with IGF-I, NGF readministration deactivated JNK in PC12 cells and prevented PCD (22).

In parallel, expression of a dominant negative mutant of c-Jun, the main substrate of JNK, inhibits apoptosis of sympathetic neurons induced by NGF deprivation (24). In T lymphocytes, overexpression of active JNK-1 induces PCD, whereas dominant negative JNK1 prevents radiation-induced apoptosis (25). Finally, there are several reports that ceramide-mediated apoptosis occurs via JNK signaling (26–28). We and others (25, 47) speculate that activated JNK allows c-Jun to serve as a pivotal mediator of cell survival. However, like that of p38 kinase, the role of JNK activation in stress-induced or cytokine-induced PCD is dependent on the specific cell and stress in question (1, 69). For example, Liu et al. (69) found that JNK plays no role in tumor necrosis factor-mediated PCD in MCF7 cells, and Virdee et al. (70) recently reported that c-Jun expression does not cause sympathetic neurons to die.

Our studies suggest a connection between IGF-I-mediated JNK inhibition and ERK downstream signaling cascades. Treatment of cells with the MEK inhibitor PD98059 blocked IGF-I signaling effects on JNK, whereas blocking PI-3K pathways had no effect. Preliminary studies from our laboratory employing a MEK1 mutant corroborate these findings. SH-SY5Y cells transfected with a dominant negative MEK1 mutant (71) display diminished IGF-I inhibition of JNK nuclear translocation. Our data agree with several reports of MEK kinases 1 and 2 activating either MKK4 or JNK itself (reviewed in Ref. 72). Furthermore, our data would also support the idea that MEK kinases interact with other family members, such as the specific mixed-lineage kinases or the p21-activated kinases to regulate MKK4 and mediate JNK activity (43). Alternatively, a recently described family of serine/threonine kinases, MAP kinase-interacting kinases 1 and 2, act downstream of both JNK and ERK and may define a convergence point between stress and growth factor activation (73).

In summary, we found that IGF-I differentially regulates p38 kinase and JNK activity under stressful conditions in SH-SY5Y cells. Our results, along with those of others, suggest that the contributions of different signaling pathways to cell function are both cell-specific and growth factor-specific. Finally, our results support the evolving concept that cell survival and death are determined by a dynamic balance of p38 kinase, JNK, and ERK activity.

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