Two Different Signal Transduction Pathways Are Implicated in the Regulation of Initiation Factor 2B Activity in Insulin-like Growth Factor-1-stimulated Neuronal Cells*

Received for publication, January 11, 2000, and in revised form, March 9, 2000
Published, JBC Papers in Press, April 6, 2000, DOI 10.1074/jbc.M000238200

Celia Quevedo‡§, Alberto Alcázar‡¶, and Matilde Salinas

From the Servicio de Bioquímica-Investigación, Hospital Ramón y Cajal, 28034 Madrid, Spain

Eukaryotic initiation factor eIF-2B plays an important role in translation regulation and has been suggested to be implicated in the increased protein synthesis promoted in response to growth factors. We have used primary cultured neurons to delineate the signaling pathways by which insulin-like growth factor-1 (IGF-1), which plays a critical role in the survival of neuronal cells, promotes eIF-2B and protein synthesis activation. Treatment of cortical neurons with IGF-1 (100 ng/ml) for 30 min stimulates [3H]methionine incorporation, and a parallel increase in eIF-2B activity was observed. Wortmannin and LY294002 reversed both effects, indicating that phosphatidylinositol 3-kinase mediates IGF-1-induced protein synthesis and eIF-2B activation. IGF-1 induced glycogen synthase kinase 3 (GSK-3) inactivation in a phosphatidylinositol 3-kinase-dependent fashion because it is inhibited by wortmannin and LY294002. By using GSK-3 immunoprecipitated from untreated and IGF-1-treated cells, we demonstrate the phosphorylation of eIF-2B coincident with its inactivation. The treatment of cortical neurons with IGF-1 also promoted the activation of mitogen-activated protein kinase (MAPK). The MAPK-activating kinase (MEK) inhibitor PD98059 inhibited MAPK activation and reversed IGF-1-induced protein synthesis and eIF-2B activation. These findings suggest that IGF-1-induced eIF-2B activation on neurons is promoted through phosphatidylinositol 3-kinase and GSK-3 kinase, and we report an IGF-1-induced MEK/MAPK activation pathway implicated in eIF-2B activation.

Translational control is pivotal in the intracellular action by which growth factors promote their effects. Protein synthesis is activated in different cell types by a variety of growth factors, which are determinant for cell growth, differentiation, and survival. The pathway through which this mechanism is exerted is not well characterized, at least in neurons of the central nervous system. Translational control starts at the level of initiation (1, 2). The polypeptide chain initiation depends on initiation factor 2 (eIF-2), which is required for ternary complex formation (eIF-2GTP-Met-tRNAi), as well as the binding of mRNA to the ribosomes and recognition of the initiator codon, and constitutes the steps subject to the finest regulation. The eIF-2 factor is required for priming each 40 S ribosomal subunit for every round of translation (3–5), but upon 80 S initiation complex formation, GTP is hydrolyzed and eIF-2 is released from the ribosome as a binary complex (eIF-2-GDP), which is functionally inactive. Initiation factor 2B (eIF-2B) promotes eIF-2 recycling by catalyzing the dissociation of GDP from eIF-2-GDP in the presence of GTP (4, 6), making eIF-2 available to undergo a further round of initiation.

eIF-2B activity can be regulated in different ways. Firstly, phosphorylated eIF-2 in the α subunit (eIF-2α) is a competitive inhibitor of eIF-2B activity (7). eIF-2B activity can be regulated in the absence of changes in phosphorylated eIF-2α (eIF-2αP) levels; thus allosteric effectors such as NADPH that binds to eIF-2B are necessary to maintain nucleotide exchange activity in vitro (8). Moreover, eIF-2B activity can be regulated in vitro by phosphorylation of its ε subunit. Three kinases have been described that are able to phosphorylate eIF-2B in vitro the ε subunit of eIF-2B (eIF-2Be), casein kinases 1 and 2, and glycogen synthase kinase-3 (GSK-3) (9, 10). eIF-2Be phosphorylation by casein kinases 1 and 2 enhances eIF-2B activity, whereas phosphorylation by GSK-3, which requires previous eIF-2Be phosphorylation, has an inhibitory effect (11–13).

The down-regulation of translation caused by eIF-2α phosphorylation and consequent inhibition of eIF-2B activity has been established as a general mechanism for cellular response to different stress situations (14, 15). Changes in eIF-2B activity in vitro, in the absence of modified eIF-2αP levels, have been observed in diverse cell types as a response to different treatments (16–21). Parallel decreases in casein kinase 2 and eIF-2B activities (22), as well as increased eIF-2B activity coincident with GSK-3 inactivation have been reported in response to nerve and epidermal growth factors (21). Insulin acutely stimulates protein synthesis in mammalian cells, and several translation factors are regulated in response to insulin, including eIF-2B (23). eIF-2B activation by insulin, studied only in non-neuronal cells, depends upon GSK-3 inactivation by a mechanism that involves its phosphorylation at conserved residues (Ser9 in GSK-3β and Ser21 in GSK-3α) (23, 24), with this effect being mediated by phosphatidylinositol 3-kinase.

* This work was supported by Grant PM97-0071 from the Spanish Ministry of Education and Science and Grant 97/2114 from the Funds for Research on Health Sciences, Spanish Ministry of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Serv. Bioquímica-Investigación, Hospital Ramón y Cajal, Ctra. Colmenar km 9.1, 28034 Madrid, Spain. Tel.: 34-91-336-9016; Fax: 34-91-336-9016; E-mail: alberto.alcazar@hrc.es.

‡ These authors contributed equally to this work.

§ Recipient of a fellowship from Madrid Autonomous Government.

¶ These authors contributed equally to this work.

1 The abbreviations used are: eIF-2, eukaryotic initiation factor 2; eIF-2α, a subunit of eIF-2; eIF-2αP, phosphorylated eIF-2α subunit; eIF-2B, eukaryotic initiation factor 2B; eIF-2Be, ε subunit of eIF-2B; ERK, extracellular signal-regulated kinase; GSK-3, glycogen synthase kinase-3; IGF-1, insulin-like growth factor-1; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK-activating kinase; PAGE, polyacrylamide gel electrophoresis; PI3-K, phosphatidylinositol 3-kinase; PKB, protein kinase B, also known as Akt.
(PI3-K) (25). Recent findings have shown that the direct link between PI3-K activation and GSK-3 inactivation is provided by protein kinase B (PKB), which is located downstream of PI3-K and phosphorylate GSK-3 at the serine regulatory sites (26). So far, this signaling pathway has been found to be independent of mitogen-activated protein kinase (MAPK) (21, 24, 25, 27).

Insulin-like growth factor-1 (IGF-1) plays a crucial role in growth and cell development regulation and exerts its action by activating multiple signal transduction pathways, notably Ras-MAPK and PI3-K pathways (28–31). IGF-1 promotes survival of central nervous system neurons, where the PI3-K pathway via PKB activation seems to be critical and where the activation of MEK/MAPK pathway fails or has not been described (29, 31–33). So far, the possible role of eIF-2B in IGF-1-mediated response of neurons has not been established.

We have investigated the effect of IGF-1 on protein synthesis and eIF-2B activities in primary cultures of cortical neurons. Furthermore, by using specific inhibitors of PI3-K and MEK (MAPK/extracellular signal-regulated kinase-activating kinase) and studying GSK-3 and MAPK, we have delineated the two signal transduction pathways involved in eIF-2B activation. In the present paper, we first report two new findings. First, our findings provide the link between IGF-1-induced cellular growth and protein synthesis and eIF-2B activation. Secondly, we present evidence for the involvement of both PI3-K and MEK signaling pathways in IGF-1-dependent eIF-2B activation on neuronal cells.

Experimental Procedures

Materials—IGF-1, wortmannin, anti-ERK1 and 2, and anti-ERK1 and 2 diphosphorylated were provided by Sigma, PD-98059 was from Biomol, Leibovitz L-15, Ham’s F-12, and high glucose Dulbecco’s medium were from Life Technologies, Inc., and [3H]GDP and [γ-32P]ATP were from Amersham Pharmacia Biotech. Synthetic peptides were supplied by Chiron Technologies, anti-GSK-3 antibody was from Transduction Laboratories, and anti-phospho-GSK-3 Ser and Thr were supplied by Biomen. eIF-2 and eIF-2B were purified from calf brain (34).

Primary Neuronal Cultures—Primary cultures of cells from cerebral cortex were obtained from 16-day-old fetuses removed from timed pregnant Harlan Sprague-Dawley rats. Fetuses were placed in Leibovitz L-15 medium for brain dissection. The cerebral cortex was separated from the rest of the brain using iridectomy scissors, and the meningeal membranes were carefully removed. The resulting pieces were then dissociated using Pasteur pipette and 20–21G needles into a homogeneous cell suspension. Trypan blue exclusion was used to count the live cells. Neurons were seeded on plastic multieishes precoated with 0.05 mg/ml poly-D-lysine at a density of 2–5 × 10⁵ cells/cm² and cultured at 37 °C with 5.5% CO₂ in air, in Dulbecco’s medium with 15% fetal calf serum. After 24 h, cultures were placed and maintained in serum-free medium Dulbecco’s/Ham’s F-12 (1:1, v/v) (D/F medium) supplemented with 1.8 mg/ml glucose, 100 µg/ml transferrin, 100 µg/ml putrescine, 20 mM progesterone, and 30 mM sodium selenite. 6–7-day-old neurons in culture were used in the experiments. The neuronal content, as determined by immunocytochemistry with antibodies against the neuron-specific protein β-tubulin isotype III, was found to be more than 90%. Cells were maintained in D/F medium without supplements for 16 h before treatments and then placed in the same medium in the absence or presence of additives. When inhibitors were used, cells were treated with the inhibitors for 1 h before and during the treatment. Cells were washed with ice-cold phosphate-buffered saline before harvesting.

Protein Synthesis Rate Measurement—Protein synthesis rate was assessed in 16-mm multieishes where the medium was aspirated and replaced with 0.25 ml of fresh medium containing 0.35 Ci/ml [3H]methionine (115 µCi/ml). After incubation, the dishes were washed twice with phosphate-buffered saline, and the cells were harvested in 0.25 ml of 0.25% Nonidet P-40 in buffer 20 mM Tris-HCl, pH 7.6, 10 mM potassium acetate, 1 mM dithiothreitol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidin and centrifuged for 30 min at 12,000 × g. The protein present in the supernatant was precipitated with 10% trichloroacetic acid, and radioactivity was determined by liquid scintillation.

eIF-2B Activity Measurement—Both untreated and treated cells cultured on 35-mm multieishes were lysed for 10 min in hypotonic buffer (10 mM Tris-HCl, pH 7.6, 10 mM KCl, 1 mM dithiothreitol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 10 µg/ml leupeptin, pepstatin, and antipain, 2 mM β-glycerophosphate, 2 mM sodium molybdate, and 0.2 mM sodium orthovanadate). The lysate was made to 4% magnesium acetate and 140 mM potassium acetate and was centrifuged for 10 min at 12,000 × g. All the steps were carried out at 4 °C. A binary complex eIF-2B-[3H]GDP was formed as described (35). eIF-2B activity present in cell extracts (60 µg of protein) was measured for its capacity to exchange eIF-2-bound [3H]GDP for free GDP (35). 1 pmol of eIF-2B-[3H]GDP was used as a substrate, and the GDP exchange reaction was incubated for 5 min. eIF-2B activity was expressed as a percentage or pmol of [3H]GDP released from binary complex.

IGF-1 was used as a positive control and eIF-2B activity present in cell extracts cultured on 35-mm multieishes were lysed, harvested in 10% urea, 5% 2-mercaptoethanol, 5 mM sodium phosphate, 70 mM sodium chloride (10 × 10⁶ cells/ml), and kept for 30 min at room temperature. The cell lysate was then centrifuged at 18,500 × g for 30 min, and the supernatant was resolved in horizontal isoelectric focusing slab gels. The bands corresponding to eIF-2α and eIF-2δ proteins were stained on immunoblots with a polyclonal antibody (1:100) purified by immunoadfinity and quantified as described (36) with an image analyzer with software package (Diversitex, PDI, New York). Quantification of bands was as described above.

GSK-3 activity and eIF-2B phosphorylation determinations—Cell extracts (10 µg), prepared in the same way as that for eIF-2B assay, from both untreated and treated cells were assayed for GSK-3 activity in 50 mM Tris-HCl, pH 7.5, 2 mM MgCl₂, 100 µM ATP, 1 µCi of [γ-32P]ATP (0.5 Ci/mmol), with 12 µg of RAAEELSDRAGFQL eIF-2B-based peptide used as a substrate, or with 12 µg of RAAEELSDRAGFQL as a negative control as described (37), in a volume of 25 µl. After 15 min at 30 °C, 10 µl of the sample was spotted onto P81 phosphocellulose paper, rinsed three times with 3% (v/v) phosphoric acid, and counted. The other 10-µl sample of the reaction mixture was analyzed by SDS-PAGE run at a pH of 7.8 (to avoid the peptide exclusion) followed by autoradiography and quantified with an image analyzer as described above.

For eIF-2B phosphorylation by GSK-3, 50 µg of cell extract was immunoprecipitated with 3 µl of anti-GSK-3 and 25 µl of protein G-Sepharose as described (38). One-half of the immunoprecipitated sample was incubated in 10 mM Hepes, pH 7.4, 0.2 mM EDTA, 10 mM MgCl₂, 25 µM ATP, 1 µCi of [γ-32P]ATP, and 1 µg of purified eIF-2B in a total volume of 40 µl. The reaction was stopped by adding 12.5 µl of SDS sample buffer and then resolved by SDS-PAGE. The dried gel was stained and exposed to film. The resulting autoradiographs were quantified as described. The other fraction was incubated in the same conditions without radioactive ATP and with 0.3 µg of purified eIF-2B and assayed for eIF-2B activity as described above.

Phospho-specific GSK-3 and MAPK Western Blots—Cell extracts, prepared in the same way that for eIF-2B assay, from both untreated and treated cells were assayed for GSK-3 activity in 50 mM Tris-HCl, pH 7.5, 2 mM MgCl₂, 100 µM ATP, 1 µCi of [γ-32P]ATP (0.5 Ci/mmol), with 12 µg of RAAEELSDRAGFQL eIF-2B-based peptide used as a substrate, or with 12 µg of RAAEELSDRAGFQL as a negative control as described (37), in a volume of 25 µl. After 15 min at 30 °C, 10 µl of the sample was spotted onto P81 phosphocellulose paper, rinsed three times with 3% (v/v) phosphoric acid, and counted. The other 10-µl sample of the reaction mixture was analyzed by SDS-PAGE run at a pH of 7.8 (to avoid the peptide exclusion) followed by autoradiography and quantified with an image analyzer as described above.

Statistical Analysis—Results are expressed as the mean ± S.E. values for independent experiments. Statistical analysis was performed using t test for paired and unpaired data versus control values or analysis of variance and Dunnett’s post-test for comparisons between treated groups.
RESULTS

IGF-1 Effect on Protein Synthesis—Cultured neurons were treated with IGF-1 for 30 min, 60 min, or 2 h, and the protein synthesis rate was determined in the presence of 0.35 Ci/mmol [3H]methionine (115 μCi). As shown in Fig. 1A, amino acid incorporation was linear ($r^2$, 0.994 both) at all the times assayed. Protein synthesis was significantly increased after exposure to 10 μg/ml insulin (not shown) and 100 ng/ml IGF-1 1.25- and 1.3-fold over control, respectively (Fig. 1A). A concentration of 0.1 μM wortmannin, a PI3-K inhibitor, was enough to block the activation of [3H]methionine incorporation produced by IGF-1 (Fig. 1B), suggesting that PI3-K pathway is necessary for protein synthesis activation. Moreover, PI3-K inhibitor LY294002 (30 μM) reduced protein synthesis rate below 0.1 μM wortmannin level. The MEK inhibitor PD98059 (30 μM) inhibited protein synthesis rate below control levels. This finding suggests a role for MEK/MAPK kinases activation in growth factor-induced protein synthesis in our cultures.

eIF-2B Activation by IGF-1—Neuronal cells were treated for 30 min with growth factors and processed to measure eIF-2B activity as described above (see “Experimental Procedures”). Insulin (10 μg/ml) increased eIF-2B activity by 32% (not shown), whereas IGF-1 (100 ng/ml) induced a 45% increase (Fig. 2) and nerve growth factor (100 ng/ml) did not have any effects on eIF-2B activity (not shown). Fig. 2A shows the linearity ($r^2$, 0.959) of the reaction between 0.5 and 1.5 pmol of substrate (binary complex, eIF-2-[3H]GDP). Wortmannin, PD98059, and LY294002 inhibited IGF-1-induced activation of eIF-2B (Fig. 2B), paralleling the effects produced on protein synthesis (Fig. 1B). The inhibitors did not produce any effects on untreated control cells or eIF-2B activity assay at the concentrations tested (not shown).

eIF-2a Phosphorylation State and eIF-2B Levels—IGF-1-induced increased eIF-2B activity, which might be due to decreased levels of their physiological inhibitor (eIF-2aP), or might be a reflection of increased cellular levels of the factor. Consequently, untreated and IGF-1-treated cells for 30 min were lysed and analyzed by horizontal isoelectric focusing slab gels and protein immunoblot to determine eIF-2a and eIF-2aP levels or by SDS-PAGE and protein immunoblot to achieve eIF-2Be levels. The bands corresponding to eIF-2a and phosphorylated eIF-2a (eIF-2aP) were identified by a polyclonal antibody recognizing both forms of eIF-2a (Fig. 3A). eIF-2B protein detection was carried out by a monoclonal antibody recognizing eIF-2Be, and the amount of eIF-2Be was considered as being representative of the total amount of eIF-2B (Fig. 3B). Quantification of the bands in three independent experiments showed no differences between untreated and IGF-1-treated cells in the phosphorylated form of eIF-2a (12.4 ± 1.3% versus 11.5 ± 0.8% of total eIF-2a) or eIF-2B levels (62.4 ± 3.8 versus 61 ± 2.1, in arbitrary units), suggesting that increased eIF-2B activity in the latter was not due to either the change in eIF-2a phosphorylation status or eIF-2B levels.
**GSK-3 Activity in IGF-1-treated Cells**—To determine GSK-3 involvement in IGF-1-induced eIF-2B activation, kinase activity was measured in untreated and IGF-1-treated neuronal cells. As shown in Fig. 4A, IGF-1 treatment induced a 31% (from 34 to 22 arbitrary units) decrease in the phosphorylation of the specific peptide used as a substrate of the reaction, which correlates with the observed activation of both eIF-2B activity and protein synthesis rate (Figs. 1 and 2). IGF-1-induced GSK-3 inactivation was blocked by wortmannin and LY294002 but not by PD98059 (Fig. 4B), indicating the known upstream regulation of GSK-3 by PI3-K. PI3-K inhibition by wortmannin and LY294002 promoted GSK-3 activation and eIF-2B and protein synthesis induction. PD98059 did not exert any effects on GSK-3 inactivation by IGF-1, suggesting the independent regulation of GSK-3 by MEK/MAP kinases.

**eIF-2B Phosphorylation by GSK-3**—To further assess the participation of GSK-3 in IGF-1 signal cascade, both phosphorylation and activity of exogenous added eIF-2B were simultaneously determined in GKS-3 immunoprecipitates from IGF-1-treated and untreated neurons. eIF-2B phosphorylation by GSK-3 from IGF-1-treated cells was 32% lower (from 17 to 12 arbitrary units) than eIF-2B phosphorylation produced by GSK-3 in untreated cells, suggesting higher activity of the enzyme in the latter (Fig. 5A). As a consequence of lower eIF-2B phosphorylation levels in IGF-1-treated immunoprecipitates, eIF-2B activity was higher in these samples (Fig. 5B). Both findings confirm that IGF-1-induced eIF-2B activation is promoted via a decrease in GSK-3-dependent eIF-2B phosphorylation.

**IGF-1 Induces GSK-3 and MAPK Phosphorylation**—By using an antibody recognizing the Ser176 residue in the phosphorylated form of GSK-3β (inactive form), and unable to detect nonphosphorylated GSK-3β (active form) we found increased inactive GSK-3 form upon stimulation of neuronal cells with IGF-1 (Fig. 6A and B, top panels). This phosphorylation was reversed by 0.1 μM wortmannin and completely blocked by 1 μM of the inhibitor (Fig. 6A). The MAP kinases ERK1 and 2 were activated by dual phosphorylation on threonine and tyrosine residues (e.g. Thr183 and Tyr185 in ERK2). Interestingly, by using an antibody against these regulatory sites of active ERK that does not recognize either the nonphosphorylated or the monophosphorylated form of the enzyme, we also detected ERK2 activity activation induced by IGF-1-treatment on neuronal cells (Fig. 6B, middle panel). As expected, PD98059 treatment did not affect GSK-3 phosphorylated status and did reverse ERK2 activation (Fig. 6B, top and middle lanes). To verify that the different lanes contained comparable amounts of sample, all immunoblots were reprobed with the corresponding antibodies recognizing both the phosphorylated and non-phosphorylated form of the kinases (Fig. 6, A and B, bottom panels). These control antibodies were raised in a different host animal to avoid signal cross-reactivity.

**DISCUSSION**

The findings reported here support the possibility that in cortical neurons two signaling components are simultaneously activated by IGF-1. Interestingly, both signal pathways, PI3-K and MEK, are implicated in protein synthesis and eIF-2B activation. The straight correlation existing between IGF-1 action and eIF-2B activation suggests eIF-2B participation in IGF-1-mediated survival described in this type of cells (29, 31, 32).

Insulin stimulates protein synthesis in mammalian cells, and at least two signaling pathways, the PI3-K and Ras-MEK/MAPK cascades, may be involved in the hormone action. Several initiation or elongation factors involved in insulin action, eIF-4E binding protein dephosphorylation, S6 ribosomal protein phosphorylation, eIF-2B activation, and eEF-2 elongation factor inactivation have been reported (23). On the contrary, in neuronal cells insulin fails to activate MAPK and, at super-physiological concentrations, promotes survival via PI3-K signaling (29). It has been postulated that in this system the effects of insulin are consequence of insulin-IGF-1 receptor cross-reactivity (39). In agreement with these results, we found
higher activation of protein synthesis and eIF-2B in IGF-1-treated than that of insulin-treated neurons.

IGF-1 effect on eIF-2B activity cannot be explain in terms of an increased factor level or decreased eIF-2aP levels. Instead, the straight correlation observed between PI3-K inhibition, GSK-activation, and eIF-2B inhibition suggests that eIF-2B activity could be regulated through phosphorylation. This mechanism has been proposed to explain eIF-2B activation in response to nerve growth factor and epidermal growth factor in PC12 cells (21). Although eIF-2B activation has been reported in vitro in different types of cells following stimulation with several treatments including growth factors, eIF-2B phosphorylation status in vivo has not been demonstrated as yet (13).

IGF-1 has become significant because it is known to promote important cellular responses that vary with the different types of cell. For instance, IGF-1 promotes hypertrophy by growth and differentiation in many types of cells (30, 40), mediates anabolic and cardiovascular actions of growth hormone in vivo (30), and induces cerebellar neuron survival (29, 31, 32). It has very recently established the important role of IGF-1 in the treatment of neurodegenerative disorders (33, 41). Our results delineate IGF-1-induced activation signaling pathway for protein synthesis in neuronal cells, which implies PI3-K activation, as shown with the inhibitors wortmannin and LY29002, phosphorylation, and consequently inactivation of GSK-3 and eIF-2B activation. Although GSK-3 is a substrate of PI3-K and has been established to mediate IGF-1 effects on neuronal survival (29). Because PKB is sensitive to wortmannin and independent of MAPK pathway (26, 27), PKB activation may be the link between PI3-K activation and GSK-3 inactivation in IGF-1-induced protein synthesis and eIF-2B activation in cortical neurons. A similar pathway has been proposed to explain nerve growth factor and epidermal growth factor-induced protein synthesis and eIF-2B activation in PC12 cells (21).

Insulin and IGF-1 have failed to activate the Ras-MEK/MAPK pathway in certain types of cells, including cortical and cerebellar neurons (29, 31–33). Our findings clearly show the existence of an IGF-1-induced increase in MAPK (ERK2) phosphorylation (active form of the enzyme), specifically inhibited by PD98059, which demonstrates the activation of this cascade. Our finding that IGF-1 activates MAPK and increases eIF-2B activation in a MEK pathway-dependent fashion, raised the possibility that MAPK regulates eIF-2B factor. The fact that the inhibition of PI3-K by wortmannin promotes GSK-3 activity, and eIF-2B and protein synthesis inhibitions, whereas PD98059 does not affect GSK-3 activity, suggests an MEK-independent regulation of GSK-3. We can conclude that IGF-1 induces protein synthesis activation and eIF-2B activity in a
PI3-K- and MEK kinase-dependent fashion.

Here we report findings supporting the possibility that IGF-1 promotes activation of two different signaling pathways in cortical neurons; interestingly both of them promote protein synthesis and eIF-2B activation. The observed parallelism between eIF-2B activation and protein synthesis suggests eIF-2B involvement in protein synthesis regulation following IGF-1 addition. IGF-1-induced eIF-2B activation occurs in a PI3-K- and MEK kinase-dependent fashion. The two pathways seem to be independent each other, because MEK inhibitor PD98059 did not affect GSK-3 activity (see above), nor did PI3-K inhibitor LY294002 affect MAPK (ERK1 and 2) phosphorylation (42), but both mechanisms are necessary to maintain eIF-2B activation. Further studies are necessary to disclose the steps implicated in MEK-induced activation of eIF-2B and to fully establish the potential role of eIF-2B in neuron survival.

Acknowledgments—We are indebted to M. Gómez-Calcerrada and J. M. Martín for kind technical and editorial assistance, respectively.

REFERENCES

1. Hershey, J. W. B., Mathews, M. B., and Sonenberg, N. (1996) Translational Control, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2. Clemens, M. J. (1996) Int. J. Biochem. Cell Biol. 31, 1–25
3. Ochoa, S. (1983) Arch. Biochem. Biophys. 223, 325–349
4. Pain, V. M. (1996) Eur. J. Biochem. 236, 747–771
5. Kimball, S. R. (1999) Int. J. Biochem. Cell Biol. 31, 25–29
6. Manchester, K. L. (1997) Biochem. Biophys. Res. Commun. 239, 223–227
7. Rowlands, A. G., Panniers, R., and Henshaw, E. C. (1988) J. Biol. Chem. 263, 5526–5533
8. Dholakia, J. N., Mueser, T. C., Woodley, C. L., Parkhurst, L. J., and Wahba, A. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 6746–6750
9. Dholakia, J. N., and Wahba, A. J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 51–54
10. Welsh, G. I., and Proud, C. G. (1993) Biochem. J. 294, 625–629
11. Singh, L. P., Denslow, N. D., and Wahba, A. J. (1996) Biochemistry 35, 3290–3212
12. Welsh, G. I., Miller, C. M., Loughlin, A. J., Price, N. T., and Proud, C. G. (1998) FEBS Lett. 421, 125–130
13. Jefferson, L. S., Fabian, J. R., and Kimball, S. R. (1999) Int. J. Biochem. Cell Biol. 31, 191–200
14. Clemens, M. J. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds) pp. 139–172, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
15. Paschen, W., and Doutreleau, J. (1999) J. Cereb. Blood F. Met. 19, 1–18
16. Welsh, G. I., and Proud, C. G. (1992) Biochem. J. 284, 19–23
17. Karinch, A. M., Kimball, S. R., Vary, T. C., and Jefferson, L. S. (1993) Am. J. Physiol. 264, E101–E108
18. Gilligan, M., Welsh, G. I., Flynn, A., Bujalska, I., Diggie, T. A., Denton, R. M., Proud, C. G., and Docherty, K. (1996) J. Biol. Chem. 271, 2121–2125
19. Welsh, G. I., Miyamoto, S., Price, N. T., Safer, B., and Proud, C. G. (1996) J. Biol. Chem. 271, 11410–11415
20. Kimball, S. R., Horetsky, L. R., and Jefferson, L. S. (1998) J. Biol. Chem. 273, 30945–30953
21. Kleijn, M., Welsh, G. I., Schepers, G. C., Voorna, H. O., Proud, C. G., and Thomas, A. A. M. (1998) J. Biol. Chem. 273, 5536–5541
22. Aroor, A. R., Singh, L. P., and Wahba, A. J. (1995) Exp. Hematol. 23, 1204–1211
23. Proud, C. G., and Denton, R. M. (1997) Biochem. J. 328, 329–341
24. Cross, D. A. E., Alessi, D. R., Cohen, P., Andjelkovich, M., and Hemmings, B. A. (1995) Nature 378, 785–789
25. Welsh, G. I., Stokes, C. M., Wang, X., Sakauye, H., Ogawa, W., Kasuga, M., and Proud, C. G. (1997) FEBS Lett. 410, 418–422
26. van Weeren, P. C., de Bruyn, K. M. T., de Vries-Smits, A. M. M., van Lint, J., and Burgering, B. M. T. (1998) J. Biol. Chem. 273, 13150–13156
27. Rhoads, R. E. (1999) J. Biol. Chem. 274, 30337–30340
28. Cross, D. A. E., Alessi, D. R., Vandenheede, J. R., McDowell, H. E., Hundal, H. S., and Cohen, P. (1994) Biochem. J. 303, 21–26
29. Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R., and Greenberg, M. E. (1997) Science 273, 661–664
30. Foncée, R., Andersson, M., Ketterman, A., Blakesley, V., Sapag-Hagar, M., Sugden, P. H., Leroith, D., and Lavandero, S. (1999) J. Biol. Chem. 272, 19115–19124
31. Miller, T. M., Tansey, M. G., Johnon, E. M. Jr., and Creedon, D. J. (1997) J. Biol. Chem. 272, 9847–9853
32. D. M. S. R., Borodetz, K., and Soloff, S. P. (1997) J. Neurosci. 17, 1548–1560
33. Heck, S., Lezoualc’h, F., Engert, S., and Behl, C. (1999) J. Biol. Chem. 274, 9828–9835
34. Alcázar, A., Martín, M. E., Seria, E., Rodríguez, S., L. Fando, J., and Salinas, M. (1995) J. Neurochem. 65, 754–761
35. Alcázar, A., Rivera, J., Gómez-Calcerrada, M., Muiñoz, F., Salinas, M., and Fando, J. L. (1996) Mol. Brain Res. 38, 101–108
36. Alcázar, A., Martín de la Vega, C., Bázán, E., Fando, J. L., and Salinas, M. (1997) J. Neurochem. 69, 1703–1708
37. Welsh, G. I., Patel, J. C., and Proud, C. G. (1997) Anal. Biochem. 244, 16–21
38. van Lint, J., Khandelwal, R. L., Merlevede, W., and Vandenheede, J. R. (1993) Anal. Biochem. 208, 332–337
39. Schumacher, R., Søns, M. A., Schlessinger, J., Brandenburg, D., Siddle, K., and Ulrich, A. (1993) J. Biol. Chem. 268, 1087–1094
40. Lavandero, S., Foncée, R., Pérez, V., and Sapag-Hagar, M. (1998) FEBS Lett. 422, 183–196
41. Torres-Aleman, I., Barrios, V., and Berciano, J. (1998) Neurology 50, 722–776
42. Vilahos, C. J., Matter, W. F., Hui, K. Y., and Brown, R. F. (1994) J. Biol. Chem. 269, 5241–5248
Two Different Signal Transduction Pathways Are Implicated in the Regulation of Initiation Factor 2B Activity in Insulin-like Growth Factor-1-stimulated Neuronal Cells

Celia Quevedo, Alberto Alcázar and Matilde Salinas

J. Biol. Chem. 2000, 275:19192-19197.
doi: 10.1074/jbc.M000238200 originally published online April 6, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M000238200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 18 of which can be accessed free at http://www.jbc.org/content/275/25/19192.full.html#ref-list-1