Glutamate Acting on N-Methyl-d-aspartate Receptors Attenuates Insulin-like Growth Factor-1 Receptor Tyrosine Phosphorylation and Its Survival Signaling Properties in Rat Hippocampal Neurons*

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Impairing intracellular signaling induced by survival factors and excess glutamate have recently been suggested to play important role in neurodegenerative processes. However, the underlying mechanism(s) and interrelationships between these factors mostly remain to be established. In the present study, we show that glutamate attenuates the tyrosine phosphorylation of the insulin-like growth factor-1 (IGF-1) receptor and the survival effect of IGF-1 (100 nM) in hippocampal cultured neurons. Pretreatment of cultured hippocampal neurons with glutamate concentration dependently inhibited the tyrosine phosphorylation of IGF-1 receptors as well as that of IRS-1 and Shc, two IGF-1 receptor adapter proteins. The effect of glutamate was also evident on the phosphorylation of Akt, as well as its upstream kinase PI3K/PDK1 and downstream targets, GSK3β and FOXO3a. The inhibitory effect of glutamate (1 mM) was blocked by antagonists of the N-methyl-D-aspartate (NMDA) receptor, including MK801 (20 μM) and AP5 (100 μM), but not by blockers of other ionotropic or metabotropic glutamate receptor sub-types demonstrating the involvement of the NMDA receptor. This hypothesis is supported further by the observation that treatment with NMDA concentration dependently inhibited the activation and phosphorylation of IGF-1 receptors and downstream targets induced by IGF-1 (100 nM). These findings demonstrate that glutamate can block the effect of IGF-1 by decreasing IGF-1 receptor signaling and responsiveness, hence attenuating the survival properties of this trophic factor in neuronal cells. Our results also suggest a novel mechanism by which glutamate can reduce cell viability and induce neurotoxicity.

Insulin-like growth factor-1 (IGF-1) 2 is a trophic factor with multiple biological functions, including important roles during development, and in the maintenance of cellular integrity throughout the organism (1, 2). Various studies have demonstrated the existence of both IGF-1 and IGF-1 receptors (IGF-1Rs) in different brain regions, including the hippocampal formation, a region known to play important roles in learning processes and that is severely affected in Alzheimer disease (2, 3). IGF-1 possesses trophic effects in the hippocampus and promotes survival of cultured hippocampal neurons against various insults (3–8). Consistent with its neuroprotective role, levels of IGF-1 are up-regulated following various brain insults supporting the notion that IGF-1 is a key neuroprotective/neurorescuing factor (3, 9).

The biological effects of IGF-1 are mostly mediated by type I IGF receptors. Binding of IGF-1 to this receptor activates its intrinsic receptor tyrosine kinase, which subsequently phosphorylates several intracellular substrates such as the insulin receptor substrate-1 (IRS-1) and Shc (8, 10–11), leading to the activation of various signaling pathways, including mitogen-activated protein kinase (MAPK) (also called extracellular signal-regulated kinase; ERK) but preferentially the phosphatidylinositol 3-kinase (PI3K)/Akt (1, 2, 9) pathway. Akt is a serine/threonine kinase and a downstream target of PI3K involved in cell survival induced by various growth factors, including IGF-1 (2, 12–14). The enzymatic phosphorylation of the Thr-308 and Ser-473 residues activates Akt (15–18), which, in turn, phosphorylates and inhibits several pro-apoptotic proteins such as Bad (19), GSK3β (20), caspase-9 (21), and most recently the winged-helix family of transcription factors known as FOXOs (14, 22, 23), leading to cell survival (2, 13, 22).

Glutamate is the major excitatory neurotransmitter in the mammalian brain, responsible for basal excitatory synaptic transmission and many forms of synaptic plasticity such as long term potentiation and long term depression associated with cognitive processes. The various effects of glutamate are mediated by specific receptors belonging to two major families, namely ionotropic and metabotropic receptors (24, 25). Ionotropic glutamate receptors are ligand-gated ion channels consisting of three subtypes known as AMPA (α-amino-3-hydroxy-

mitogen-activated protein kinase; NMDA, N-methyl-D-aspartate; PDK1, phosphatidylinositol 3,4,5-triphosphate-dependent kinase 1; PDK2, phosphatidylinositol 3,4- triphosphate-dependent kinase 2; PIIK, phosphatidylinositol 3-kinase; PKC, protein kinase C; GSK3β, glycogen synthase kinase 3β; MEK, MAPK/ERK kinase; mGlu, metabotropic receptor; CPCC0Et, 7-hydroxymethylpentofuranosyl [b] chiron-1a-carboxylic acid ethyl ester; DNQX, 6,7-Dinitroquinoxaline-2,3-dione.
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5-methyl-1-4-isoxazoxazole propionate), NMDA (N-methyl-d-aspartate), and kainate receptors on the basis of the affinity of their preferential agonist (24, 25). Metabotropic (mGlu) receptors are G-protein-coupled receptors divided into three main classes. Group I mGlu receptors are coupled to phospholipase C and intracellular Ca2+ signaling, whereas group II and III receptors are negatively coupled to adenyl cyclase and the production of cAMP. Preferential metabotropic receptor agonists include the excitatory amino acid analogue L-quisqualic acid (group I), DCG-IV (group II), and L-AP4 (group III).

Glutamate receptors are essential for the normal functioning of the brain. However, their excessive activation can lead to neuronal damage ranging from acute hypoxic-ischemic brain injuries to chronic neurodegenerative diseases (24, 25). Interestingly, it has been suggested that excessive glutamate levels can block the activation of Akt, and hence alter cell survival, linking glutamate excitotoxicity to impaired cell survival signaling (26). However, it is not clear from this earlier study if trophic factor signaling pathways are involved. We report here that glutamate is able to attenuate the survival promoting effects of IGF-1 by interrupting IGF-1R survival signaling. The effect of glutamate is mediated by the NMDA receptor sub-type as mimicked by NMDA itself and blocked by an antagonist, MK-801. Taken together, these data reveal a novel mechanism by which glutamate and NMDA receptors regulating trophic factor signaling may be involved in a variety of neurotoxic events leading to neurodegenerative diseases.

EXPERIMENTAL PROCEDURES

Materials—Human recombinant IGF-1 (San Francisco, CA). Anti-rat C-terminal IRS-1, anti-Pi3K p85, and anti-phosphotyrosine (4G10) were purchased from Upstate Biotechnology Inc (Lake Placid, NY). Anti-phospho-IGF-IR (1165/1166), Anti-phospho-IGF-IR (1161), Anti-IGF-IR (1165/1166), Anti-IGF-IR (1161), anti-phospho-SHP-2,Tyr542), anti-phospho-SHP-2 (Tyr580), anti-SHP-2 (Tyr542), anti-phospho-Akt-Ser308 were obtained from Signalway (Pearland, TX) via Seaject Scientific (Beijing, China), anti-phospho-AktThr473, anti-phospho-AktY, anti-phospho-Src, anti-phospho-PDK1, anti-phospho-GSK3β, anti-phospho-PKcα, anti-phospho-PKcβ, anti-phospho-MEK, and anti-phospho-Akt or MAPK were from Cell Signaling (Beverly, MA); anti-Foxo3a/FKHLR1; anti-phospho-Foxo3a/FKHLR1Thr32, and anti-phospho-Foxo3a-FKHLR1Ser253 were purchased from Upstate Biotechnology (Lake placid, NY). Monoclonal anti-IGF-1R (ab-1) was from Oncogene Research Products (Cambridge, MA); anti-Akt, anti-MAPK, anti-IRS-1, anti-IGF-1R β subunit, anti-NMAD2, anti-NMAD3, anti-phosphotyrosine (PY99), and all secondary antibodies conjugated with horseradish peroxidase were from Santa Cruz Biotechnology (Santa Cruz, CA). MK801, AP-5, DNQX, NMDA, LY 341495, and 7-hydroxyiminocyclopropan [b] chromen-1a-carboxylic acid ethyl ester (CPCCOEt) were from Tocris Bioscience. 96-well shuttle rat neuron Nucleofector™ kits were purchased from Amaz (Cologne, Germany). Human CMV-SHP-2-Myc-His cDNA was from Fulengen (Guangzhou, China), siRNA1–4 for SHP-2 were purchased from Shanghai GenePharma Co. (Shanghai, China). Glutamate and cell culture reagents were purchased from Invitrogen, whereas all other reagents were from Sigma or Fisher Scientific.

Hippocampal Neuronal Cultures—Hippocampal cultured neurons were prepared as described by Zheng et al. (27) with minor modifications and from fetuses (embryonic day 20) of pregnant Sprague-Dawley rats (Charles River Breeding Laboratories, St. Constant, Quebec, Canada). Animal care was according to protocols and guidelines approved by McGill University Animal Care Committee and the Canadian Council for Animal Care. Hippocampi were dissected in Ca2+- and Mg2+-free Hanks’ balanced salt solution (HBSS) supplemented with 15 mM HEPES, 10 units/ml penicillin, and 10 µg/ml streptomycin. Tissues were collected and washed 4 or 5 times with HBSS and then submitted to an enzymatic digestion at 37 °C with 0.25% trypsin in HBSS media for 10 min. The reaction was stopped by the addition of fetal bovine serum (final concentration 10%), and tissue was rinsed with HBSS 4–5 times to remove fetal bovine serum. The cellular suspension was then obtained by repeating aspirations through a pasteur pipette. Following a centrifugation at 800 × g for 10 min, the medium was removed and cells were resuspended in a chemically defined serum-free Neurobasal medium supplemented with 2% B27, 20 µM L-glutamine, 15 mM HEPES, 10 units/ml penicillin, and 10 µg/ml streptomycin. Neurons were plated at density of 5–8 × 105 cells/ml in culture plates (coated with 10 µg/ml poly-D-lysine) under serum-free conditions and grown at 37 °C with 5% CO2 humidified atmosphere. On the day following the plating, the medium was replaced with fresh culture medium. Medium was changed again with either the medium as above or Neurobasal supplemented with 1% N2 after 4–5 days. Experimental treatments were performed on the seventh day after plating.

Treatments—Before each experiment, the culture medium was replaced with Neurobasal 2 h before adding the desired reagents. To study the signaling pathways induced by IGF-1 in cultured hippocampal neurons, cells were treated with 100 nM IGF-1 for 6–10 min. To study the effect of glutamate, neurons were pretreated for 1 h with various concentrations of glutamate or NMDA before exposure to IGF-1 as above. Alternatively, cells were first exposed to MK801 (20 µM, 20 min), AP-5 (50 µM, 20 min), 6,7-Dinitroquinoxaline-2,3-dione (DNQX) (10 µM, 20 min), LY 341495 (300 nM, 20 min), and CPCCOEt (100 µM, 20 min) followed by a pretreatment with glutamate and then stimulation with 100 nM IGF-1. All experiments were repeated at least three to four times.

Transfection of Cultured Hippocampal Neurons with SHP-2 and siRNA for SHP-2 by Nucleofector™—Primary hippocampal neurons from fetuses (embryonic day 20) were prepared as above and the transfections of SHP-2, and its siRNA was performed by Nucleofector (Amaxa, Germany) with 96-well shuttle rat neuron Nucleofector kit according to the manufacturer’s instructions.

Western Blotting—Western blotting was performed as described earlier with some modifications (2, 27). Briefly, treated cells from different experimental conditions were rinsed twice with ice-cold HBSS and lysed in either sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% w/v SDS, 10% glycerol, 50 mM dithiothreitol, and 0.1% w/v bromphenol blue) or RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Igepal CA-630, 0.1% SDS, 50 mM NaF, 1 mM NaVO3, 5 mM
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RESULTS

Glutamate Attenuates the Tyrosine Phosphorylation of IGF-1 Receptors Induced by IGF-1 and IGF-1-promoted Survival Effects in Cultured Hippocampal Neurons—Impaired IGF-1 intracellular signaling and excess glutamate have been suggested to be involved in neurodegenerative processes (24). To investigate the effects of exposure to glutamate on IGF-1R signaling and its survival properties, cultured hippocampal neurons were pretreated with 1 mM glutamate, and the tyrosine phosphorylation of IGF-1Rs and the survival effects of IGF-1 were determined (Fig. 1). IGF-1 caused the tyrosine phosphorylation of IGF-1R (Fig. 1, A and B) and protected cultured hippocampal neurons from B27 deprivation-induced cell death (Fig. 1C). Pretreatment with glutamate (1 mM) significantly attenuated the tyrosine phosphorylation of IGF-1Rs (Fig. 1, A and B) and the survival effects of IGF-1 (Fig. 1C).

Glutamate Decreases Tyrosine Phosphorylation of IRS-1, Shc, and PI3K—We studied next the effect of glutamate on IGF-1R downstream signaling. Fig. 2A shows that glutamate significantly blocked the phosphorylation of Shc- and IRS-1, two main adaptor proteins of the IGF-1R and that of PI3K, a main downstream signaling protein of IGF-1 (Fig. 2A). Moreover, the effect of glutamate in the tyrosine phosphorylation of the IGF-1R and its adaptor protein IRS1 was clearly concentration-dependent (Fig. 2B).

Glutamate Attenuates IGF-1-induced Activation of the PI3K/Akt Pathway While Promoting the Phosphorylation of IGF-1-stimulated MAPK—We have previously shown that the PI3K/Akt pathway is essential for the survival effects of IGF-1, whereas the MAPK pathway plays a rather minimal role (27, 28). Accordingly, we studied the comparative effect of a pretreatment with glutamate on IGF-1-induced activation of the PI3K/Akt and MAPK pathways. Consistent with results in Fig. 2A showing that a pretreatment with glutamate (1 mM) attenuated the tyrosine phosphorylation of PI3K, it also inhibited the activation of PDK1 (an upstream kinase of Akt), the phosphorylation of both Thr-308 and Ser-473 of Akt, and the phosphorylation of GSK3β (Fig. 3) and FOXO3a (Fig. 4). In contrast, the same treatment enhanced IGF-1-induced activation of MAPK and its upstream kinase MEK. Glutamate (1 mM), by itself, had a minor effect on the Akt pathway while more powerfully stimulating the phosphorylation of MEK and MAPK (Fig. 3). In addition to Akt and MAPK pathways, a pretreatment with glutamate (1 mM) also attenuated the activation of different iso-

phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 50 μg/ml aproginin). Samples with equal amounts of protein were then separated by 4–20% PAGE, and the resolved proteins were electrotransferred to Hybond-C nitrocellulose. Membranes were incubated with 5% nonfat milk and 2% bovine serum albumin in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.2% Tween 20) for 1 h at room temperature and incubated overnight with appropriate primary antibody at 4 °C. The membranes were then washed twice with TBST and probed with anti-rat/mouse/rabbit/goat-horseradish peroxidase at room temperature for 1 h. Membranes were finally washed several times with TBST to remove unbound secondary antibodies and visualized using the ECL detection kit (Amersham Biosciences). A part of the SDS gel was stained with Coomassie Blue to ensure the use of equal amounts of proteins. The respective phosphorylation of IGF-1R/Akt/PDK/FKHRL1/Src/GSK3β/MAPK/PKCp/ PKCβ, and PKCγ/PKCδ was determined by Western blot using anti-phospho-IGF-

IR(1165–1166)/Akt, Akt/PDK/FKHRL1/Src/GSK3β/MAPK/PKCp/ PKCβ, and PKCδ antibodies. Blots were subsequently stripped and reprobed with antibody for respective protein or anti-β-actin to confirm equal protein loading.

Determination of Tyrosine Phosphorylation of IGF-1 Receptor, IRS-1, Shc, and PI3K by Immunoprecipitation—Cultured hippocampal neurons were treated as described above except that exposure to IGF-1 was 8 min. Cells were then rinsed with cold phosphate-buffered saline twice and collected. After centrifugation at 1000 × g for 5 min at 4 °C, cell pellets were lysed on ice in pre-cold RIPA buffer for 20 min. Cell lysates were then pelleted at 13,000 × g for 10 min, and the concentration of protein in each sample was determined using the Bio-Rad dye-

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cofactor of Akt and MAPK pathways. Consistent with results in Fig. 3, and FOXO3a (Fig. 4). In contrast, the same treatment enhanced IGF-1-induced activation of MAPK and its upstream kinase MEK. Glutamate (1 mM), by itself, only had a minor effect on the Akt pathway while more powerfully stimulating the phosphorylation of MEK and MAPK (Fig. 3). In addition to Akt and MAPK pathways, a pretreatment with glutamate (1 mM) also attenuated the activation of different iso-

FIGURE 1. Glutamate attenuates the tyrosine phosphorylation of IGF-1Rs and the survival/protective effect of IGF-1 in cultured hippocampal neu-

rons. Following pretreatment with 1 mM glutamate, cultured hippocampal neurons were exposed to 100 nM IGF-1 for 8 min (for tyrosine phosphorylation of IGF-1R) and 2–3 days (for survival assay) and the tyrosine (ty) phosphorylation of IGF-1R (A), densitometric analysis (B), and cell viability (C), were determined as described under “Experimental Procedures.” Glutamate inhibited the tyrosine phosphorylation of IGF-1R (A and B) and the protective effect of IGF-1 in hippocampal neurons (C). Data represent assays from at least three independent experiments.

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forms of PKC, including PKCα, PKCβ, PKCγ, and PKCδ, and had a minor effect on Src kinase (Fig. 3).

The Inhibitory Effect of Glutamate on IGF-1 Receptor Signaling Is Mediated by the NMDA Receptor—Knowing that glutamate is able to attenuate the IGF-1R/PI3K/Akt signaling survival pathway, we examined next the role of various glutamate receptor sub-types on this effect. Cultured hippocampal neurons were pretreated with the selective NMDA receptor antagonists MK801 or AP-5 (two selective NMDA receptor antagonists), or DNQX (antagonist for the non-NMDA receptor, AMPA, and kainate receptors), CPCCOEt (selective, non-competitive mGluI antagonist), and LY 341495 (mGluII/III antagonist), and the effect of glutamate (1 mM) on IGF-1R phosphorylation and the PI3K/Akt pathway was determined. Only the NMDA receptor antagonists MK801 and AP-5 significantly inhibited the effects of glutamate (A). MK801 also reversed the effect of glutamate on the activation of PI3K/Akt pathway induced by IGF-1 (B). Blots represent prototypical examples of experiments replicated at least three times.

FIGURE 2. Glutamate reduces the tyrosine phosphorylation of IGF-1R substrate proteins IRS-1, Shc, and PI3K in cultured hippocampal neurons. Cultured hippocampal neurons were pretreated with 1 mM glutamate for 1 h, and then exposed to 100 nM IGF-1 for 8 min, and the tyrosine (ty) phosphorylation of IRS-1, Shc, and PI3K was determined as described under “Experimental Procedures.” As shown in A, IGF-1 dramatically increased the tyrosine phosphorylation of these proteins, whereas a pretreatment with glutamate significantly inhibited the effect of IGF-1, B; the effect of glutamate on the tyrosine phosphorylation of IGF-1Rs and IRS-1 is concentration-dependent. Blots represent prototypical examples of experiments replicated at least three times.

FIGURE 3. Pretreatment with glutamate inhibits IGF-1-induced activation of the PI3K pathway while enhancing the effect of IGF-1 on the MAPK pathway. Cultured hippocampal neurons pretreated with glutamate (1 mM) were stimulated with 100 nM IGF-1, and the activation/phosphorylation of various kinases was determined as described under “Experimental Procedures.” Pretreatment with glutamate significantly attenuated the phosphorylation (ty) of proteins of the PI3K pathway, including PDK1, Akt, GSK3β, as well as PKC kinase while enhancing the phosphorylation of MAPK and its upstream kinase, MEK. Blots represent prototypical examples of experiments replicated at least three times.

FIGURE 4. NMDA receptor antagonists block the effect of glutamate on the tyrosine phosphorylation of the IGF-1R and the PI3K/Akt pathway. Cultured hippocampal neurons were pretreated with the NMDA receptor antagonists MK801 or AP-5 (two selective NMDA receptor antagonists), or DNQX (antagonist for the non-NMDA receptor, AMPA, and kainate receptors), CPCCOEt (selective, non-competitive mGluI antagonist), and LY 341495 (mGluII/III antagonist), and the effect of glutamate (1 mM) on IGF-1R phosphorylation and the IGF-1R/Akt pathway was determined. Only the NMDA receptor antagonists MK801 and AP-5 significantly inhibited the effects of glutamate (A). MK801 also reversed the effect of glutamate on the activation of PI3K/Akt pathway induced by IGF-1 (B). Blots represent prototypical examples of experiments replicated at least three times.
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**FIGURE 5.** NMDA concentration dependently blocks IGF-1R signaling in hippocampal neurons. Cultured hippocampal neurons were pretreated with 0.3 or 1 mM NMDA and then stimulated with 100 nM IGF-1, and the tyrosine (ty) phosphorylation of the IGF-1R and Akt was determined as described under “Experimental Procedures.” NMDA concentration dependently attenuates the phosphorylation (ty) of the IGF-1R and of Akt (p). Blots represent prototypical examples of experiments replicated at least three times.

**FIGURE 6.** MK801 blocks the inhibitory effect of glutamate and NMDA on IGF-1-mediated survival in cultured hippocampal neurons. Cultured hippocampal neurons were pretreated with various selective glutamate subtype receptor antagonists MK801, DNQX, CPCCOEt, and LY 341495, and the effect of glutamate (0.3 mM) and NMDA (1 mM) on IGF-1 (100 nM)-mediated survival was determined as described under “Experimental Procedures.” Only MK801 significantly blocked the effects of glutamate and NMDA on IGF-1-mediated survival as measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Data represent means ± S.E. from at least three independent experiments.

siRNA for SHP-2 Attenuated the Effect of Glutamate in Hippocampal Neurons Enhanced Its Effect—To further confirm the role of SHP-2, hippocampal neurons were transfected with a mixture of four siRNA for SHP-2 using Nucleofector technology. Fig. 8 shows that siRNA for SHP-2 inhibited the expression of SHP-2 and attenuated the effect of glutamate on the phosphorylation of IGF-1R.

**DISCUSSION**

In the present study, we showed that glutamate, at concentrations not inducing excitotoxicity, is able to attenuate the survival effects of IGF-1. Glutamate acted by impairing IGF-1R coupling and signaling to the PI3K/Akt/FOXO pathway while possibly promoting IGF-1 action on the MAPK pathway. The effects of glutamate are mediated by the NMDA receptor subtype.

Glutamate Inhibits the Survival Effect of IGF-1 in Hippocampal Neurons—The survival effect of IGF-1 was attenuated by a treatment with glutamate at concentrations as low as 100 μM. At such low concentrations, glutamate, by itself, had no apparent effect on cell survival suggesting that its effects were not due to excitotoxicity but to the inhibition of the survival properties of IGF-1. Our data also suggest that cell death inducers, such as glutamate, are not only able to activate pathways leading to cell death, but can also inhibit survival pathways hence amplifying their effects. Comparable findings have been reported from another group (37) and for tumor necrosis factor-α, a pro-inflammatory cytokine known to mediate various forms of experimental neurodegeneration and to induce insulin/IGF-1 resistance in various tissues (30–33). Similar results were also obtained with prion proteins, ethanol, and mercury (34–37).

Glutamate Impairs the Survival Signaling of IGF-1 at the Level of the IGF-1 Receptor—Exposure to glutamate significantly blocked the survival effects of IGF-1 by reducing IGF-1R

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**FIGURE 7.** Glutamate inhibits the survival effect of IGF-1 in hippocampal neurons. Cultured hippocampal neurons were pretreated with various selective glutamate subtype receptor antagonists MK801, DNQX, CPCCOEt, and LY 341495, and the effect of glutamate (0.3 mM) and NMDA (1 mM) on IGF-1-mediated survival was determined as described under “Experimental Procedures.” Only MK801 significantly blocked the effects of glutamate and NMDA on IGF-1-mediated survival as measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Data represent means ± S.E. from at least three independent experiments.
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Signaling. This hypothesis is based on the following experimental results. First, glutamate, concentration dependently inhibited the tyrosine phosphorylation of the IGF-1R and of its adaptor protein IRS-1. Second, glutamate reduced the phosphorylation of PI3K and PDK1 (an upstream kinase of Akt), leading to reduced Akt activity via reduced phosphorylation. This event results in the activation of death effectors such as FOXO3a by reducing their phosphorylation. Third, the glutamate treatment preferentially affected the PI3K/Akt over the MAPK pathway. Hence, a pre-exposure to glutamate can reduce IGF-1 survival effect by directly altering IGF-1R phosphorylation and signal transduction. Consistent with our results, a recent report showed that glutamate was able to activate the tyrosine phosphatase SHP-2 in cerebellar neurons, which enhanced the association of SHP-2 with TrkB, leading to the inhibition of the phosphorylation of this receptor and diminished trophic properties (29). In accordance with these findings, preliminary data also showed that glutamate is able to attenuate the phosphorylation of Akt induced by brain-derived neurotrophic factor in hippocampal neurons.3

NMMDA Receptors Mediate the Effect of Glutamate on IGF-1 Receptor Signaling and Survival—Specific antagonists of the various glutamate receptors were used to establish which subtype was responsible for the effects of glutamate on IGF-1R signaling and survival. Our data demonstrate that the NMDA receptor subtype likely plays a key role. Indeed, only the NMDA receptor antagonists MK801 and AP-5 inhibited the effects of glutamate on the tyrosine phosphorylation of IGF-1R, and the phosphorylation of IGF-1R was determined as described under “Experimental Procedures.” siRNA inhibited the expression of SHP-2 and attenuated the inhibitory effect of glutamate on the phosphorylation of IGF-1R. Blots represent prototypical examples of experiments replicated at least three times.

3 W. Zheng and R. Quirion, unpublished results.
acting on NMDA receptors likely activates downstream signaling kinases that phosphorylate serine residues on IGF-1Rs leading to altered receptor tyrosine phosphorylation (38). Alternatively, glutamate could activate phosphatases inducing IGF-1R dephosphorylation. The first hypothesis appears rather unlikely, because glutamate decreased the phosphorylation of classic PKC (this study). PKCs have been shown to be involved in the phosphorylation of serine residues of the IGF-1R in other cells (38). Consistent with this observation, the inhibition of MAPK, a downstream effector of PKC, had no effect on the action of glutamate on IGF-1R signaling, although glutamate seems to enhance the activation of MAPK kinase. Further studies will be required to establish the functional significance of the effect seen here on the MAPK pathway.

Phosphatases may be better candidates to explain the effects observed in the present study. Indeed, it has recently been shown that the activation of the tyrosine phosphatase SHP-2 can dephosphorylate TrkB (29). SHP-2 may also be involved in the phosphorylation of the IGF-1R (39–40). Can dephosphorylate TrkB (29). SHP-2 may also be involved in the phosphorylation of the IGF-1R (39–40). Interestingly, glutamate-activated SHP-2 could dephosphorylate TrkB (29). SHP-2 may also be involved in the phosphorylation of the IGF-1R (39–40). This hypothesis is supported by the following results: (a) glutamate stimulated the phosphorylation of SHP-2; (b) knocking down the expression of SHP-2 by siRNA attenuated the inhibitory effect of glutamate on the phosphorylation of IGF-1R induced by IGF-1; and (c) the tyrosine phosphatase inhibitor, sodium vanadate, blocked the effect of glutamate on IGF-1-induced phosphorylation of IGF-1R and Akt.

In summary, the present study reveals that glutamate, acting via the NMDA receptor sub-type, is capable of impairing IGF-1R signaling and survival effects in hippocampal neurons. These findings suggest a novel mechanism by which glutamate can regulate neuronal viability by altering trophic factor receptor signaling.

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