In the central nervous system, Zn\(^{2+}\) is concentrated in the cerebral cortex and hippocampus and has been found to be toxic to neurons. In this study, we show that exposure of cultured cortical neurons from mouse to increasing concentrations of Zn\(^{2+}\) (10–300 \(\mu\)M) induces a progressive decrease in global protein synthesis. The potency of Zn\(^{2+}\) was increased by about 2 orders of magnitude in the presence of Na\(^{+}\)-pyrithione, a Zn\(^{2+}\) ionophore. The basal rate of protein synthesis was restored 3 h after Zn\(^{2+}\) removal. Zn\(^{2+}\) induced a sustained increase in phosphorylation of the \(\alpha\) subunit of the translation eukaryotic initiation factor-2 (eIF-2\(\alpha\)), whereas it triggered a transient increase in phosphorylation of eukaryotic elongation factor-2 (eEF-2). Protein synthesis was still depressed 60 min after the onset of Zn\(^{2+}\) exposure while the state of eEF-2 phosphorylation had already returned to its basal level. Moreover, Zn\(^{2+}\) was less effective than glutamate to increase eEF-2 phosphorylation, whereas it induced a more profound inhibition of protein synthesis. These results suggest that Zn\(^{2+}\)-induced inhibition of protein synthesis mainly correlates with the increase in eIF-2\(\alpha\) phosphorylation. Supporting further that Zn\(^{2+}\) acts at the initiation step of protein synthesis, it strongly decreased the amount of polyribosomes.

The transition metal Zn\(^{2+}\) is widely but heterogeneously distributed in the brain. It is mainly detected in glutamatergic neurons of the neocortex and in nerve terminals of hippocampal mossy fibers (1). In these latter nerve terminals, Zn\(^{2+}\) appears to be contained in synaptic vesicles and is released with glutamate during neuronal activity (2, 3). Several studies suggest that Zn\(^{2+}\) may modulate excitatory neurotransmission. Zn\(^{2+}\) inhibits glutamate uptake into glial cells (4) and synaptosomes (5) and facilitates \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid (AMPA) receptor-mediated neuroexcitation (6). Alternatively, Zn\(^{2+}\) has been shown to inhibit N-methyl-D-aspartate (NMDA) receptor function through both voltage-dependent and voltage-independent mechanisms (7, 8). Beside its modulatory effects on glutamatergic transmission, Zn\(^{2+}\) has been found to contribute to neuronal loss induced by transient cerebral ischemia (9). The precise mechanism responsible for the neurotoxic effect of Zn\(^{2+}\) is unknown. However, NMDA receptor antagonists appear to exert a protecting effect against Zn\(^{2+}\)-induced neurotoxicity (10).

At the intracellular level, Zn\(^{2+}\) can interact with a large variety of factors including metallothioneins, reduced glutathione, and ion transporter enzymes such as Na\(^{+}\)/K\(^{+}\)-ATPase and Ca\(^{2+}\)-ATPase (for review, see Ref. 11). In neurons, Zn\(^{2+}\) treatment and NMDA receptor stimulation lead to an inhibition of cell respiration and thus of ATP synthesis. However, Zn\(^{2+}\) inhibits the cell respiratory chain by blocking the initial step of respiration, i.e. the electron transfer between ubiquinone and cytochrome \(b\) (complex III) (12), whereas glutamate induces a loss of the mitochondrial potential by opening the transition pore (13, 14).

We have previously demonstrated that glutamate, by stimulating NMDA- and AMPA-gated channels, depresses global protein synthesis in cultured cortical neurons from the mouse (15). This effect appears to result from the phosphorylation of eukaryotic elongation factor-2 (eEF-2) by eEF-2 kinase, a Ca\(^{2+}\)-calmodulin-dependent enzyme (15). It has also been reported that Zn\(^{2+}\) inhibits protein synthesis in reticulocyte lysate by a distinct mechanism, which involves the phosphorylation of the \(\alpha\) subunit of the eukaryotic initiation factor-2 (eIF-2\(\alpha\)) (16). The aim of the present study was to determine whether Zn\(^{2+}\) depresses protein synthesis in living cortical neurons and to investigate the mechanism involved in this process.

**EXPERIMENTAL PROCEDURES**

**Primary Cultures of Cortical Neurons**—Primary neuronal cultures were prepared as described previously (17). Briefly, cortices were removed from 15-day-old Swiss mouse embryos (Iffa Credo, Lyon, France) and cells were seeded on 6- or 12-well culture dishes (3 \(\times\) 10⁵ cells/well containing 3 and 1 ml of culture medium, respectively), coated successively with poly-L-ornithine (15 \(\mu\)g/ml, \(M_0\) = 40,000, Sigma) and culture medium containing 10% fetal calf serum (Durcher, Brumath, France). The culture medium included a 1:1 mixture of Dulbecco’s modified Eagle’s medium and F-12 nutrient (Life Technologies, Inc., Paris, France), supplemented with glucose (33 mM) glutamine (2 mM), NaHCO\(_3\) (13 mM), HEPES buffer (5 mM, pH 7.4), penicillin-streptomycin (5 IU/ml and 5 \(\mu\)g/ml, respectively), and a mixture of salt and hormones containing insulin (25 \(\mu\)g/ml), transferrin (100 \(\mu\)g/ml), progesterone (20 ng/ml), putrescine (60 \(\mu\)g/ml), and Na\(_2\)SeO\(_3\) (30 ng/ml). Cells were maintained for 6 days at 37 °C in a humidified atmosphere containing 8% CO\(_2\) without medium change. In these conditions, the cultures were shown to be highly enriched in neurons by immunocytochemistry using an anti-microtubule-associated protein 2 monoclonal antibody (IgG1, Biomakor, Israel). Less than 7% of the cells exhibited immunoreactivity with a rabbit antibody raised against glial fibrillary acid protein ( Dakopatts, Glostrup, Denmark) (data not shown).

**Measurement of \([^{35}\text{S}]\text{Methionine}\) and \([^{3}H]\text{Leucine}\) Incorporation**—
Neurons grown in 12-well culture dishes were washed twice in 1 ml of HEPES buffer (in mM: HEPES, 20; glucose, 5.5; NaCl, 120; KCl, 5.5; MgCl₂, 0.9; CaCl₂, 1.1; pH 7.4) and then incubated for 30 min in this medium in the presence of drugs and 50 μM either methionine or leucine. [³⁵S]methionine (1000 Ci/mmol, Amersham Pharmacia Biotech) or [³H]leucine (159 Ci/mmol, Amersham Pharmacia Biotech) were added (4 μCi/μl each) during the last 10 min of the incubation period. The labeling was stopped by washing in 1 ml of ice-cold phosphate buffered saline and addition of 1 ml of cold trichloroacetic acid (10%, w/v). Cells were scraped, and centrifugations were performed for 10 min at 10,000 × g. Amino acid uptake into neurons and incorporation into proteins was estimated by counting the radioactivity in the supernatant and the pellet, respectively. Results are expressed as the ratio between the radioactive amino acid incorporated into proteins (trichloroacetic acid-precipitable fraction) and the radioactive amino acid taken up into the cells (supernatant).

Detection of Intracellular Zn²⁺ in Cortical Neurons—Intracellular Zn²⁺ was detected in neurons grown on glass slides using the Zn²⁺-selective and membrane-permeant fluorescent dye N-(6-methoxy-quinolyl)-p-toluenesulfonamide (TSQ). Glass slides were placed in a superfusion chamber where cells were superfused with HEPES buffer. Neurons were exposed to drugs including TSG (0.001%, wt/v, prepared from a stock solution of 0.5% in dimethyl sulfoxide) using a multichannel superfusion device. The superfusion chamber was placed on the stage of a Nikon Diaphot inverted microscope equipped with a 75-watt xenon light and a 40× epifluorescence oil immersion objective. Light was filtered at 360 nm with a 10-nm-wide interference filter and emission light was passed through a 380-nm dichroic long pass filter (barrier 420 nm). Images were acquired with an intensified CCD camera and digitized using an Argus 50 interface (16 video frames per digitized image, allowing the recording of 1 image/s). The camera and the digitizing system were from Hamamatsu (Japan). The camera dark noise was subtracted from the recorded crude image at the beginning of each experiment.

Analysis of eIF-2α Phosphorylation—Cortical neurons grown in six-well culture dishes were labeled for 3.5 h with [³²P]orthophosphate (200 μCi/ml) in 1 ml of HEPES buffer. Drugs were then added to cells for the indicated times. Neurons were lysed in 200 μl of immunoprecipitation buffer containing 100 mM NaCl, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 50 mM NaF, 50 mM β-glycerophosphate, 1 mM benzamidine, 0.5 mM Na⁺-vanadate, 1% Triton X-100, and a protease inhibitor mixture (Roche Molecular Biochemicals), and centrifuged for 10 min at 10,000 × g. Supernatant proteins (100 μg) were immunoprecipitated overnight with an antibody recognizing specifically eIF-2α (10 μg of purified immunoglobulin per sample) and 30 μl of protein A-Sepharose beads. The serum recognizing eIF-2α was obtained by immunization of rabbits with a synthetic peptide derived from the sequence of the protein (LSKRRVSPEAKIC) and purified on an affinity column. The purified antibody recognized an unique band around 36 kDa in whole homogenates prepared from cultured cortical neurons (data not shown). Immunocomplexes were washed three times in immunoprecipitation buffer, boiled in SDS loading buffer (18), and resolved on 10% polyacrylamide gels. Incorporation of [³²P] into eIF-2α was detected by autoradiography and quantified by PhosphorImager. The amount of immunoprecipitated eIF-2α was estimated in an aliquot of each samples by Western blotting using a monoclonal antibody directed against eIF-2α (20).

eIF-2α phosphorylation state was also analyzed by sequential immunoblotting, first with an antibody recognizing specifically the phosphorylated form of eIF-2α (1/250 dilution, Research Genetics Inc., Huntsville, AL) and then with the polyclonal antibody recognizing total eIF-2α (1/20,000 dilution). Neurons, grown in six-well culture dishes, were exposed to drugs in HEPES buffer for the indicated times. Incubations were stopped by replacing the medium by 0.3 ml of boiling SDS (1%, w/v), in order to prevent protein dephosphorylation by phosphatases. Protein concentration was determined with a bichinonic acid method (19), using bovine serum albumin as standard. Samples containing 50 μg of protein were resolved on 10% polyacrylamide gels and transferred to nitrocellulose. Antibody-antigen complexes were detected with an enhanced chemiluminescence method (Renassiance kit from NEN Life Science Products) using a horseradish peroxidase-coupled donkey anti-rabbit secondary antibody (Amersham Pharmacia Biotech).

Analysis of eEF-2 Phosphorylation—The phosphorylation of eEF-2 in living cortical neurons was analyzed by sequential immunoblotting first with an antibody that specifically recognized eEF-2 phosphorylated on Thr56 (1/1,000 dilution) and then with an antibody recognizing eEF-2 independently of its phosphorylation state (1/1,000 dilution) (15), as described above.

RESULTS

Zn²⁺ Inhibits Protein Synthesis in Cortical Neurons—The exposure of cultured cortical neurons to Zn²⁺ for 30 min resulted in a marked decrease in [³H]leucine (Fig. 1) or [³⁵S]methionine (data not shown) incorporation into proteins. This inhibition of neuronal protein synthesis was concentration-dependent (IC₅₀ = 51 ± 7 μM, mean ± S.E. of values obtained in five independent experiments performed in triplicate), and the potency of Zn²⁺ was increased (1–10 μM range, Fig. 1) in the presence of 20 μM Na⁺-pyrithione, a Zn²⁺ ionophore. As detected using the Zn²⁺-sensitive fluorescent dye TSQ, the Na⁺-pyrithione treatment strongly increased the staining of neurons exposed to 3 μM Zn²⁺ (Fig. 2). It should be noted that, at this low concentration (3 μM), Zn²⁺ inhibited by 50% the protein synthesis in the presence of Na⁺-pyrithione, whereas it was ineffective in its absence. None of these treatments (Zn²⁺ with or without Na⁺-pyrithione) significantly altered the uptake of radioactive amino acids into neurons (data not shown).

Role of Glutamate-operated Channels and Voltage-gated Ca²⁺ Channels in Zn²⁺-induced Inhibition of Protein Synthesis—Zn²⁺ influx into neurons has been shown to occur through AMPA- or NMDA-gated channels and L-type voltage-dependent Ca²⁺ channels (24). However, neither nifedipine, an antagonist of L-type voltage-gated Ca²⁺ channels, nor (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801) or 6,7-dinitroquinoxaline-2,3-dione (DNQX), antagonists of NMDA and AMPA receptors, respectively, suppressed the inhibition of protein synthesis induced by...
100 μM Zn^{2+} (Fig. 3). Additional experiments were performed to determine whether these treatments modify the Zn^{2+}-induced TSQ fluorescence. Since TSQ binds Zn^{2+} in a saturable process, variations of Zn^{2+} bound to TSQ must be investigated in the presence of Zn^{2+} concentrations that are largely lower than those leading to the saturation of the dye (used at 30 μM). Thus, in order to warrant absence of saturation of the dye, we have investigated the mechanisms of Zn^{2+} entry in neurons exposed to 3 μM Zn^{2+} (see Fig. 2). In this experimental condition, the co-application of DNQX and nifedipine with Zn^{2+} did not decrease TSQ fluorescence (Fig. 4). Only MK-801 decreased by 38 ± 7% (n = 33 cells tested) the fluorescence signal of the dye.

**Time Course and Reversibility of Zn^{2+}-induced Inhibition of Protein Synthesis**—A progressive decline of protein synthesis was observed when cortical neurons were continuously treated with 100 μM Zn^{2+}, the maximal inhibition being reached after a 50-min exposure (Fig. 5). When neurons were exposed to 100 μM Zn^{2+} for 30 min only, the marked decrease of protein synthesis already measured at the end of this treatment was followed by an almost complete recovery to control levels 3 h after the removal of Zn^{2+} (Fig. 5). This recovery was accelerated (less than 1 h) by adding the Zn^{2+} chelator, N,N,N′,N′-tetraakis(2-pyridyl-methyl)ethylenediamine (TFEN, 10 μM), immediately after the removal of Zn^{2+} (Fig. 5). TSQ fluorescence following Zn^{2+} removal declined very slowly (92 ± 7% of initial TSQ fluorescence was still detected 1 h after Zn^{2+} removal). Addition of TFEN just after Zn^{2+} removal lead to the almost complete disappearance of TSQ fluorescence in less than 2 min (data not shown).

**Possible Mechanisms Involved in the Zn^{2+}-induced Inhibition of Protein Synthesis**—We have previously demonstrated that Ca^{2+} influx in cortical neurons resulting from the activation of ionotropic glutamate receptors inhibits protein synthesis. This effect was correlated with the increase in the phosphorylation of eEF-2 by eEF-2 kinase, a Ca^{2+}-calmodulin-dependent enzyme (15). This Ca^{2+}-mediated process is likely not the main mechanism responsible for Zn^{2+}-induced inhibition of protein synthesis. Indeed, after a 1-h exposure of cortical neurons to Zn^{2+}, eEF-2 was not phosphorylated, whereas protein synthesis was still depressed (Figs. 5 and 6). A significant increase in eEF-2 phosphorylation was detected after a 30-min exposure of neurons to 100 μM Zn^{2+} (Fig. 6) but this phosphorylation was of lower amplitude than that evoked by a maximally effective concentration of glutamate (100 μM, Fig. 6), which only decreased by 50% the rate of protein synthesis in cortical neurons (15). Moreover, the exposure of neurons to 100 μM Zn^{2+} resulted in a strong decrease of RNA associated with the polyribosomal fraction (Fig. 7), indicating that the initiation but not the elongation step of protein synthesis is likely involved in Zn^{2+}-induced inhibition of protein synthesis.

Exposure of cortical neurons to a Ca^{2+}-free buffer or to thapsigargin, two treatments which are known to deplete intracellular Ca^{2+} stores, led to a marked decrease in protein synthesis (Figs. 3 and 8). It has been demonstrated in other cell types that these treatments increase the phosphorylation of eIF-2α (25, 26). Similarly, exposure of neurons to Ca^{2+}-free buffer or to thapsigargin increased the incorporation of 32P into eIF-2α by 2.6-fold (data not shown) and 4.1-fold (Fig. 8, a and c), respectively. As shown in rabbit reticulocyte lysate, heavy metals including Zn^{2+} increase the phosphorylation level of eIF-2α, a process that could account for their ability to inhibit protein translation (16). Similarly, the incorporation of 32P into eIF-2α was enhanced when neurons were exposed to 100 μM Zn^{2+} for 30 min (Fig. 8, a and c). The enhanced phosphorylation of eIF-2α in neurons exposed to Zn^{2+} or thapsigargin was also...
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FIG. 5. Time dependence and reversibility of Zn\(^{2+}\)-induced inhibition of protein synthesis. \[^{3}\text{H}]\text{Leucine incorporation was measured in cortical neurons exposed to 100 \(\mu\text{M}\) ZnCl\(_2\) for increasing times (left panel). For this purpose, \[^{3}\text{H}]\text{Leucine was added only for the last 10 min of the treatment. On the right panel, cortical neurons were transiently (30 min) exposed to 100 \(\mu\text{M}\) ZnCl\(_2\) (horizontal bar). The heavy metal chelator TPEN (10 \(\mu\text{M}\), open symbols) was added just after Zn\(^{2+}\) withdrawal. \[^{3}\text{H}]\text{Leucine was added for the last 10 min of the incubation period. Values are means \pm S.E. of data obtained in two experiments, each performed in triplicate on different cultures. TCA, trichloroacetic acid.}

FIG. 6. Effects of glutamate and Zn\(^{2+}\) on the phosphorylation of eEF-2 in cortical neurons. Cortical neurons grown in six-well culture dishes were exposed to sham treatment, glutamate (Glu, 100 \(\mu\text{M}\)) or ZnCl\(_2\) (100 \(\mu\text{M}\)) for the indicated times. Cells were harvested in boiling SDS, and proteins (50 \(\mu\text{g} \text{/lane}) were resolved on 8\% SDS-polyacrylamide gels and transferred onto nitrocellulose sheets. Immunoblotting was performed with the antibody recognizing specifically eEF-2 phosphorylated on Thr-56 and the antibody recognizing eEF-2 independently of its phosphorylation state. Immunoreactive bands were detected with a horseradish peroxidase-coupled secondary antibody, chemiluminescence, and autoradiography. Illustrated data are representative of three experiments, each performed on different sets of cultured neurons.

FIG. 7. Effect of Zn\(^{2+}\) on the amount of polyribosome in cortical neurons. Cortical neurons were exposed to sham treatment or 100 \(\mu\text{M}\) ZnCl\(_2\) for 30 min. Polyribosomal RNA were isolated as described under “Experimental Procedures.” Top, revelation of polyribosomal RNA by ethidium bromide staining. Bottom, quantification of the fluorescence intensities of 28S and 18S bands (expressed as percentage of basal). *p < 0.01, Student’s t test.

FIG. 8. Effects of Zn\(^{2+}\) and thapsigargin on eIF-2\(\alpha\) phosphorylation and protein synthesis in cortical neurons. a, cortical neurons grown in six-well culture dishes were labeled with \[^{32}\text{P}\]orthophosphate (8500 Ci/mmole, 200 \(\mu\text{Ci} \text{/well}) for 3.5 h in HEPES-buffered solution. ZnCl\(_2\) (100 \(\mu\text{M}\)) and/or thapsigargin (Thapsi, 10 \(\mu\text{M}\)) were added for an additional 30-min period. Cells were harvested in immunoprecipitation (IP) buffer, and proteins (100 \(\mu\text{g} \text{/sample}) were immunoprecipitated with a polyclonal antibody recognizing eIF-2\(\alpha\). Immunocomplexes were resolved on 10\% polyacrylamide gels and incorporation of \(^{32}\text{P}\) into eIF-2\(\alpha\) was detected by autoradiography. The amount of immunoprecipitated eIF-2\(\alpha\) was assessed in each sample by immunoblotting with a monoclonal antibody recognizing eIF-2\(\alpha\), as eIF-2\(\alpha\) phosphorylation was detected by immunoblotting with the antibody recognizing specifically phospho-eIF-2\(\alpha\) and the polyclonal antibody reacting with total eIF-2\(\alpha\). c, incorporation of \(^{32}\text{P}\) into eIF-2\(\alpha\) was quantified by PhosphorImager. Data are the means \pm S.E. of values obtained in three experiments, each performed in triplicate on different sets of cultured neurons. *p < 0.01 from basal (analysis of variance followed by Dunnett’s test).

DISCUSSION

In this study, we show for the first time that the transient exposure of living cortical neurons to Zn\(^{2+}\) markedly decreases protein synthesis. This effect was observed with concentrations of Zn\(^{2+}\) (10–300 \(\mu\text{M}\)) that are in the range of those reached in the extracellular space under physiopathological conditions such as cerebral ischemia (2).

The Zn\(^{2+}\)-induced inhibition of protein synthesis probably results from the interaction of this heavy metal with intracellular components, as it was enhanced in the presence of the Zn\(^{2+}\) ionophore, Na\(^{+}\)-pyrithione. This protein synthesis inhibi-
tion appears to be slowly reversible, as a recovery to the basal level of radioactive amino acid incorporation into proteins was observed 3 h after the transient exposure (30 min) of cortical neurons to Zn$^{2+}$. The time course of this recovery process could reflect the slow dissociation rate of Zn$^{2+}$ from its intracellular binding sites. Indeed, Zn$^{2+}$ is known to be tightly bound to several intracellular components such as phospholipids (binding to phosphate head groups) (27), membrane-bound enzymes such as phospholipases (reaction with sulfhydryl groups leading to the formation of stable mercaptides) (28), or other thiol-containing molecules such as metallothioneins or reduced glutathione (11). Supporting further this hypothesis, the Zn$^{2+}$ chelator TPEN accelerated the recovery of protein synthesis to control levels.

As already reported, Zn$^{2+}$ enters neurons by several routes including NMDA and AMPA receptors and L-type voltage-gated Ca$^{2+}$ channels (24). However, the Zn$^{2+}$-induced inhibition of protein synthesis in cortical neurons (observed in the absence of glutamatergic agonists and under non-depolarizing conditions) persisted in the presence of glutamate receptor antagonists or nifedipine, a blocker of L-type voltage-sensitive Ca$^{2+}$ channels. Similarly, DNQX and nifedipine did not alter TSQ fluorescence signal, but MK-801 partially decreased the fluorescence of the dye. However, for the aforementioned technical considerations, this set of experiments was done in the presence of a low concentration of Zn$^{2+}$ as compared with those required to observe an inhibition of protein synthesis. Assuming that MK-801 blocks with the same efficiency Zn$^{2+}$ entry in neurons exposed to 100 μM Zn$^{2+}$, one can predict from the dose response for Zn$^{2+}$ on protein synthesis that such a partial decrease of intracellular Zn$^{2+}$ concentration does not significantly modify the rate of protein translation. Together, these results suggest that the process of Zn$^{2+}$ influx into neurons that leads to the inhibition of protein synthesis is different from those previously described.

We have previously reported that the global protein synthesis in neurons can be inhibited by a Ca$^{2+}$-dependent process (15). The phosphorylation of the elongation factor eEF-2 by eEF-2 kinase, a Ca$^{2+}$-calmodulin-dependent kinase, appears to be involved in the glutamate-induced reduction of protein synthesis. This conclusion was based on the close correlation between the magnitude of protein synthesis inhibition and the level of eEF-2 phosphorylation (15). Two observations suggest that, although Zn$^{2+}$ does transiently increase eEF-2 phosphorylation, this process is likely not the main mechanism responsible for Zn$^{2+}$-induced inhibition of protein synthesis. First, a 100 μM Zn$^{2+}$ treatment, which inhibited protein synthesis to a larger extent than that induced by a maximally effective concentration of glutamate (100 μM), led to an increase in eEF-2 phosphorylation significantly lower than that induced by this excitatory amino acid. Second, protein synthesis was still depressed 60 min after the onset of Zn$^{2+}$ exposure, whereas the recovery to control levels of the phosphorylation state of eEF-2 was almost complete.

The decrease in the amount of polyribosome in neurons exposed to Zn$^{2+}$ suggests that the metal acts instead at the initiation step of protein synthesis, in which the phosphorylation of eIF-2α constitutes a key mechanism of regulation. Three kinases have been found to phosphorylate eIF-2α: the heme-regulated inhibitor of erythroid cells, the interferon-inducible RNA-dependent protein kinase (PKR), and the recently discovered PERK (for PKR-like endoplasmic reticulum kinase) (25, 26, 29–32). It has been suggested that Ca$^{2+}$ release from endoplasmic reticulum or the resulting depletion of intracellular Ca$^{2+}$ stores induces the phosphorylation of eIF-2α (25, 26, 31). As initially proposed, PKR could be involved in this phosphorylation (25, 26). However, according to two recent reports, there is strong evidence that the newly discovered eIF-2α kinase PERK is implicated in this cellular stress response (31, 32). Thus, the activation of this kinase may be responsible for both the increase in eIF-2α phosphorylation and the inhibition of protein synthesis found in neurons exposed to either thapsigargin or a Ca$^{2+}$-free medium.

Zn$^{2+}$ treatment also induced a prominent inhibition of protein synthesis associated with an increased phosphorylation of eIF-2α in cortical neurons. Due to the sensitivity of intracellular Ca$^{2+}$-sensitive fluorescent dyes to Zn$^{2+}$, it is not yet possible to determine whether Zn$^{2+}$ treatment also evokes a release of Ca$^{2+}$ from intracellular stores and therefore whether Zn$^{2+}$ acts by a mechanism similar to that of thapsigargin. However, in accord with the involvement of a common process, the effects of Zn$^{2+}$ and thapsigargin were not additive on both the increase in eIF-2α phosphorylation and the inhibition of protein synthesis. PERK activation could intervene in the unfolded protein response, which consists of the attenuation of protein synthesis rate following the accumulation of incorrectly folded proteins in the endoplasmic reticulum (31). As Zn$^{2+}$ may alter the folding of newly synthesized peptides (11), one can speculate that PERK is responsible for the increase in eIF-2α phosphorylation in response to Zn$^{2+}$, a process that could prevent further accumulation of incorrectly folded proteins in the endoplasmic reticulum. However, the increase in eIF-2α phosphorylation measured in neurons exposed to Zn$^{2+}$ may also result from the inhibition of phosphatase activity, as okadaic acid induced a similar increase in eIF-2α phosphorylation. As PERK (or PKR) activation involves an autophosphorylation process, one cannot conclude whether the okadaic acid effect results from the inhibition of the dephosphorylation of the kinase or of the initiation factor.

A complex consisting of eIF-2α, GTP, and tRNA$^{Met}$ must be formed during each cycle of translation initiation. This requires the regeneration of active eIF-2α by exchange of an eIF-2α-bound GDP for GTP, a process catalyzed by eIF-2B. The affinity of eIF-2B for the phosphorylated α-subunit of eIF-2 is 150-fold greater than for the unphosphorylated form of the protein (33) and the level of eIF-2α in the brain is 5 times higher than that of eIF-2B (34). Therefore, one might expect that if only 20% of the total amount of eIF-2α is phosphorylated, most of eIF-2B should become unavailable to catalyze the guanine nucleotide exchange on the remaining unphosphorylated pool of eIF-2α. If the relative amounts of eIF-2α and eIF-2B are in the same range in the brain and in cortical neurons, a relatively low rate of phosphorylation of eIF-2α, as detected in cortical neurons, could account for the large inhibition of protein synthesis induced by Zn$^{2+}$ treatment or following mobilization of Ca$^{2+}$ from intracellular stores.

One important issue concerns the physiological significance of the Zn$^{2+}$-induced inhibition of protein synthesis. The transient inhibition of protein translation in neurons exposed to Zn$^{2+}$ might lead to the expression of a new pattern of proteins that can be involved in specific pathological or physiological processes (35, 36).

Apoptosis, but not necrosis, is an active process requiring protein synthesis, which is thus suppressed by protein synthesis inhibitors. Accordingly, we have demonstrated that the inhibition of protein synthesis by cycloheximide or diphtheria toxin treatments (which are not toxic by themselves) protects cortical neurons from the toxicity evoked by low concentrations of NMDA (15), which are known to selectively induce an apoptotic process (37). On the contrary, the pharmacological inhibition of protein synthesis did not protect neurons against strong excitotoxicity (37). Therefore, the inhibition of protein synthe-
sis seems to constitute a self-protecting mechanism rather than an active deleterious process. However, due to the marked inhibition of protein synthesis induced by Zn\(^{2+}\), which is in the same range as those evoked by cycloheximide and diphtheria toxin treatments, it was impossible to demonstrate such a protective mechanism in Zn\(^{2+}\)-induced neurotoxicity.

Translational control in neurons could contribute to long term synaptic plasticity such as long term potentiation (LTP). The application of protein synthesis inhibitors during the induction of LTP in the hippocampus reduces its duration to 3–6 h, indicating that a critical level of protein synthesis is required for long term occurrence of LTP (38). The combined phosphorylation of eIF-2\(\alpha\) and eEF-2 following Zn\(^{2+}\) and glutamate release during LTP induction could lead to a transient inhibition of protein synthesis, allowing the establishment of a new pattern of protein expression required for the maintenance of LTP. Therefore, the phosphorylation of eIF-2\(\alpha\) by Zn\(^{2+}\) and of eEF-2 by glutamate and their convergent consequences on protein translation open new perspectives for understanding the mechanisms implicated in such an unusual co-transmission process.

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