Biochemical and Biophysical Research Communications **280,** 223–228 (2001)
doi:10.1006/bbrc.2000.4107, available online at http://www.idealibrary.com on IDEAL®

Ubiquitin-Binding Protein p62 Expression Is Induced during Apoptosis and Proteasomal Inhibition in Neuronal Cells

Erkki Kuusisto,* Tiina Suuronen,* and Antero Salminen*,†,[1]
*Department of Neuroscience and Neurology, University of Kuopio, Kuopio, Finland; and
†Department of Neurology, Kuopio University Hospital, Kuopio, Finland

Received December 6, 2000

Neuronal apoptosis is involved in several pathological conditions of the brain. Using cDNA arrays, we observed upregulation of ubiquitin-binding protein p62 expression during serum withdrawal-induced apoptosis in Neuro-2a cells. We demonstrate here that the expression levels of p62 mRNA and protein were increased in Neuro-2a cells and cultured rat hippocampal neurons by different types of proapoptotic treatments, including serum deprivation, okadaic acid, etoposide, and trichostatin A. Ubiquitin-binding protein p62 is a widely expressed cytoplasmic protein of unclear function. The ability of p62 to bind noncovalently to ubiquitin and to several signalling proteins suggests that p62 may play a regulatory role connected to the ubiquitin system. Accordingly, we show that proteasomal inhibitors MG-132, lactacystin, and PSI caused a prominent upregulation of p62 mRNA and protein expression, with a concomitant increase in ubiquitinated proteins. To conclude, p62 upregulation appears to be a common event in neuronal apoptosis. Results also suggest that the induction of p62 expression by proteasomal inhibitors may be a response to elevated levels of ubiquitinated proteins, possibly constituting a protective mechanism. © 2001 Academic Press

Key Words: **A170; programmed cell death; neuroblastoma; hippocampal neurons; neuronal degeneration; ubiquitination; Neuro-2a.**

Apoptotic neuronal death is involved in acute pathological conditions of the brain, such as ischemic stroke (1). In addition, a growing body of evidence suggests that apoptosis contributes to neuronal loss during neurodegenerative disorders, such as Alzheimer's disease (2, 3). Whereas major advances have been made in the understanding of the molecular mechanisms of apoptosis in non-neuronal contexts, in neuronal cells the mechanisms remain less well defined. In most cases, neuronal apoptosis can be blocked *in vitro* using inhibitors of mRNA or protein synthesis (4), implying that changes at the level of gene expression are necessary for the activation of the apoptotic machinery. However, only a few studies have been published in which the expression of large numbers of genes has been assayed in an attempt to identify the relevant genes.

We have used expression screening techniques, such as cDNA arrays and mRNA differential display, to identify genes induced during neuronal apoptosis (5). Here we describe the identification of ubiquitin-binding protein p62 as a gene involved in neuronal apoptosis. Although ubiquitin-binding protein p62 has recently been identified in a number of contexts (6–9), its function remains unclear. However, since p62 is able to interact noncovalently with ubiquitin and several signalling proteins, p62 may have a regulatory function by connecting signal transduction to ubiquitin-mediated proteolysis. Ubiquitin-mediated proteolysis in proteasomes is the primary mechanism for degradation of cytoplasmic proteins and has an important role in cellular signalling (10). Ubiquitin-protein conjugates are present in different types of protein aggregates associated with neurodegeneration, suggesting a dysfunction in the mechanisms involved in the removal of excessive, misfolded, or defective proteins via the ubiquitin-proteasome pathway (11). In order to clarify the possible role of p62 under conditions that involve neuronal death or accumulation of ubiquitinated proteins, we explored the expression of p62 in neuronal cell cultures subjected to apoptosis or proteasomal dysfunction. We found that p62 expression is upregulated during different *in vitro* models of neuronal apoptosis. Furthermore, we show that proteasomal inhibitors increase p62 expression, suggesting that p62

[1] To whom correspondence should be addressed at Department of Neuroscience and Neurology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland. Fax: +358-17-162-048. E-mail: antero.salminen@uku.fi.

0006-291X/01 $35.00

upregulation might be a protective response under conditions promoting protein aggregation.

MATERIALS AND METHODS

Cell culture. Mouse neuroblastoma Neuro-2a cells (CCL 131) were obtained from the American Type Culture Collection (Manassas, VA). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma, St. Louis, MO) containing 1000 mg/l D-glucose and supplemented with 10% fetal calf serum (Life Technologies, Rockville, MD), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Rat hippocampal neurons were prepared from 17-day-old Wistar rat embryos and cultured in B27-supplemented Neurobasal medium (Life Technologies) containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.5 mM L-glutamine, as described by Brewer *et al.* (12).

Treatment of cultures. Apoptosis was induced in exponentially growing Neuro-2a cells (~24 h after plating) either by withdrawal of serum or by treatment of the cells with okadaic acid (Oka, 10–30 nM), etoposide (Eto, 8 μM), or trichostatin A (TsA, 2 μM). Proteasomal inhibitors MG-132, lactacystin, and PSI were used at concentrations of 2–5 μM. Rat hippocampal neurons were treated with okadaic acid (10 nM) or lactacystin (5 μM) after 7 days *in vitro*. All drugs were purchased from Calbiochem-Novabiochem (San Diego, CA). Cells were analyzed for nuclear morphology, caspase-3 activity, and DNA integrity as previously described (13).

cDNA array hybridization. Atlas mouse cDNA expression arrays (Clontech, Palo Alto, CA) were used for the identification of genes upregulated during apoptosis. Briefly, Neuro-2a cells were cultured in serum-free medium, and total RNA was extracted from apoptotic and control cultures after 24 h. ^{32}P-labelled cDNA probes were prepared from DNase-treated RNA samples and hybridized to a pair of cDNA expression arrays according to the manufacturer's protocol. The ^{32}P signals were detected using a Storm PhosphorImager and quantified using the ImageQuaNT program (Molecular Dynamics, Sunnyvale, CA). For each detectable spot in the array pair, signal intensity ratios (i.e., apoptotic:control) were calculated and compared to the signal ratios of control genes present in the array.

Northern blotting. Total RNA was extracted from cultured Neuro-2a cells and rat hippocampal neurons using TRIzol reagent (Life Technologies). Equal amounts of RNA (10–15 μg) were denatured and resolved in an agarose/formaldehyde gel, transferred in 10 × SSC onto a Magnacharge positively charged nylon membrane (Osmonics, Westborough, MA), and fixed using a UV crosslinker (120 mJ/cm^2) (Stratalinker 2400, Stratagene, La Jolla, CA). As a control of equal loading and transfer efficiency, the blots were stained with methylene blue as described (14). A ^{32}P-labelled cDNA probe specific for p62 mRNA was prepared from the expressed sequence tag (EST) clone 576791 (RZPD, Berlin, Germany) using High Prime labelling system (Roche Molecular Biochemicals, Indianapolis, IN). The blots were prehybridized for 2 h and hybridized overnight at 65°C in a solution containing 0.5 M (with respect to Na^+) sodium phosphate, 7% SDS, 1% BSA, and 1 mM EDTA. After hybridization, the membranes were washed 2 × 15 min in 2 × SSC/0.1% SDS and 2 × 15 min in 0.1 × SSC/0.1% SDS at room temperature. The ^{32}P signals were detected and quantified using the Storm PhosphorImager and ImageQuaNT program. The results were analyzed for statistically significant differences using one-way ANOVA followed by Bonferroni's post-hoc multiple group comparison.

Immunoblotting. Neuro-2a cells were washed twice with ice-cold phosphate buffered saline (PBS) and lysed in RIPA buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 0.5% sodium deoxycholate) supplemented with 1 × Complete protease inhibitor cocktail (Roche). The lysates were stored on ice for 30 min and centrifuged at 10,000*g* for 10 min. The supernatant was used for immunoblotting. Rat hippocampal neurons were washed twice with PBS, collected in PBS using a rubber policeman, and centrifuged at 15,000*g* for 20 s. The cell pellet was lysed in PBS containing 1% Triton X-100 and 1 × Complete protease inhibitor cocktail. The lysates were stored on ice for 30 min, centrifuged at 12,000*g* for 20 min, and the supernatant was used for immunoblotting. Equal amounts of proteins (10–20 μg) were analyzed in 10% polyacrylamide–sodium dodecyl sulfate (SDS) gels according to the standard protocol (14) and transferred onto Hybond ECL (Amersham Pharmacia Biotech, Piscataway, NJ) nitrocellulose membranes using a Trans-Blot SD semi-dry blotting apparatus (Bio-Rad, Hercules, CA) according to the manufacturer's protocol. The blots were blocked in Tris-buffered saline (TBS) containing 3% nonfat dried milk and 0.05% Tween 20, and incubated with primary and secondary antibodies according to standard protocols (14). The protein bands were visualized using a chemiluminescent detection system (SuperSignal, Pierce, Rockford, IL) and Hyperfilm ECL film (Amersham Pharmacia Biotech).

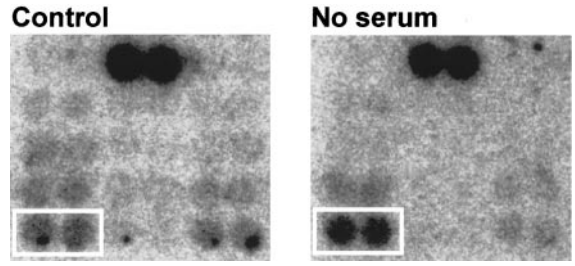

FIG. 1. Differential expression of p62 mRNA detected using Atlas mouse cDNA expression arrays. Apoptosis was induced in Neuro-2a cells by culturing the cells in serum-free medium for 24 h. Two identical arrays were hybridized with ^{32}P-labelled cDNA probes prepared from control (left) or apoptotic (right) Neuro-2a cells. Array sections containing the double spot corresponding to p62 mRNA (boxes) are shown.

Antibodies. For detection of p62 by immunoblotting, rat polyclonal antiserum raised against A170 (mouse homologue of p62; gift from Dr. T. Ishii, Univ. Tsukuba, Japan), was used at a dilution of 1:2500. Ubiquitin-protein conjugates were detected using a rabbit polyclonal antibody (Z0458; DAKO, Carpinteria, CA) using a dilution of 1:500. As secondary antibodies, anti-mouse or anti-rabbit F(ab')$_2$ fragments linked with horseradish peroxidase (Amersham Pharmacia Biotech) were used to detect the primary antibodies.

RESULTS

p62 mRNA Expression Is Upregulated during Serum Deprivation-Induced Apoptosis of Neuro-2a Cells

To identify genes induced during neuronal apoptosis, we used a cDNA array to monitor changes in mRNA expression during serum deprivation-induced apoptosis in Neuro-2a cells. Two Atlas mouse cDNA expression arrays, containing 588 cDNA fragments from known genes representing different functional classes of proteins, were hybridized with ^{32}P-labelled cDNA probes prepared from apoptotic or control RNA (Fig. 1). One of the genes that were significantly (>50% increase) upregulated in apoptotic cells was oxidative stress-induced protein A170 (GenBank Accession No. U40930), which is the mouse homologue of human ubiquitin-binding protein p62 (GenBank Accession No. NM_003900).

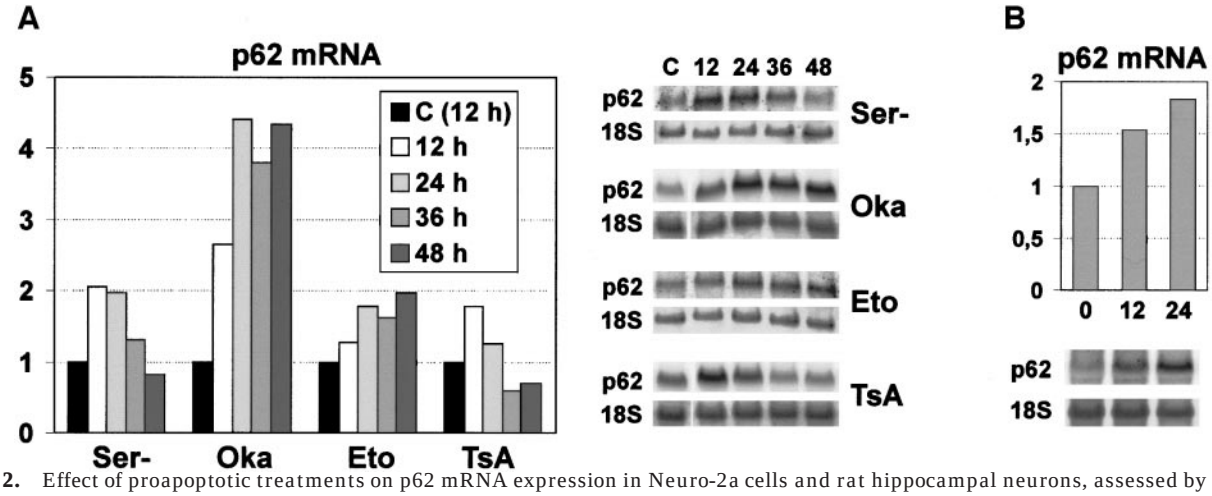

FIG. 2. Effect of proapoptotic treatments on p62 mRNA expression in Neuro-2a cells and rat hippocampal neurons, assessed by Northern blotting. (A) Neuro-2a cells were either withdrawn of serum (Ser-), or treated with okadaic acid (Oka; 30 nM), etoposide (Eto; 8 μM), or trichostatin A (TsA; 2 μM) and cultured for 12–48 h (C, untreated control, 12 h). (B) Rat hippocampal neurons were treated with okadaic acid (10 nM) after 7 days *in vitro* and cultured for 0–24 h. Values on the vertical axes are normalized pixel volumes (control = 1.0) of ^{32}P signals. As a loading control, 18S rRNA was stained with methylene blue.

Several Types of Proapoptotic Treatments Increase Level of p62 mRNA in Neuro-2a Cells and Rat Hippocampal Neurons

The present finding that the expression of p62 is induced in serum-deprived Neuro-2a cells suggested, for the first time, that the function of p62 might be related to apoptosis. In order to test the possibility that p62 is upregulated during neuronal apoptosis, irrespective of the type of apoptotic inducer, we studied p62 mRNA expression using different neuronal *in vitro* models of apoptosis (Fig. 2). In Neuro-2a cells, all treatments tested, i.e., serum deprivation, okadaic acid, etoposide, and trichostatin A, were found to increase the level of p62 mRNA markedly during 12–48 h, with varying time courses (Fig. 2A). We have previously confirmed that these treatments cause apoptotic cell death in Neuro-2a cells (13, 15). These results demonstrate that the upregulation of p62 mRNA appears to be a common feature of Neuro-2a apoptosis.

We subsequently studied whether a similar induction of p62 expression occurs during apoptosis of primary cultured neurons. In okadaic acid treated rat hippocampal neurons, p62 mRNA level was markedly increased during 12–24 h (Fig. 2B), demonstrating that the upregulation of p62 is not limited to Neuro-2a neuroblastoma cells.

Proapoptotic Treatments Increase Expression of p62 Protein in Neuro-2a Cells and Rat Hippocampal Neurons

Next we examined whether the upregulation of p62 mRNA by certain apoptotic inducers was accompanied by an increase in the protein level of p62 (Fig. 3). In immunoblot analysis, the A170 antiserum recognized a 62-kDa protein, the level of which was upregulated in Neuro-2a cells deprived of serum or treated with okadaic acid for 12−36 h (Figs. 3A and 3B). Similarly, in cultured rat hippocampal neurons, okadaic acid in-

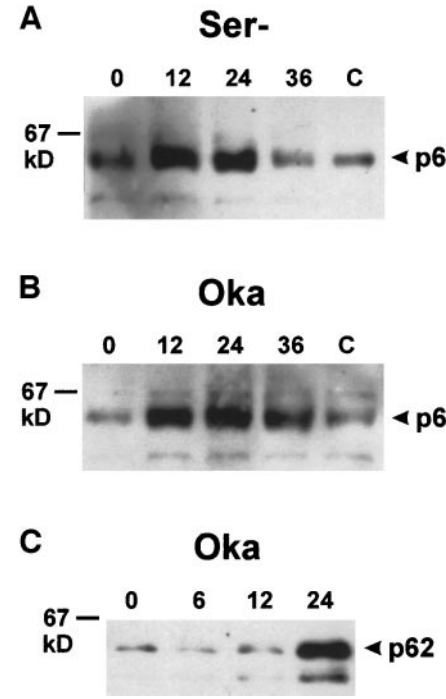

FIG. 3. Induction of p62 protein expression by pro-apoptotic treatments in Neuro-2a cells and rat hippocampal neurons. Neuro-2a cells were (A) withdrawn of serum or (B) treated with okadaic acid (30 nM) and cultured for 0–36 h (C, untreated control, 36 h). (C) Rat hippocampal neurons were treated with okadaic acid (10 nM). Levels of p62 protein were analyzed by immunoblotting, using p62-specific rabbit antiserum.

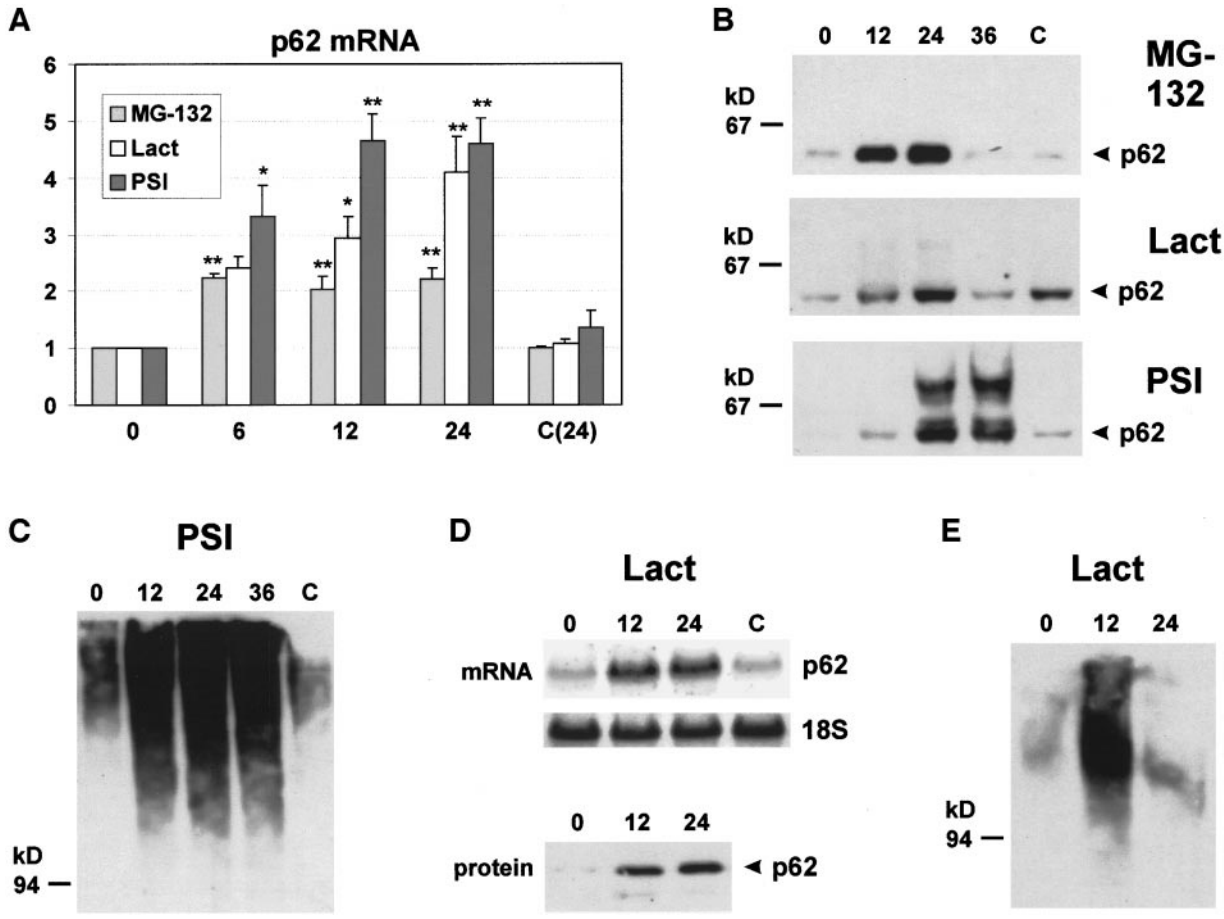

FIG. 4. Effect of proteasomal inhibitors on the levels of p62 mRNA, p62 protein, and ubiquitinated proteins in Neuro-2a cells (A–C) and rat hippocampal neurons (D, E). Cells were treated with 2–5 μM of MG-132, lactacystin (Lact), or proteasome inhibitor I (PSI) as indicated. (A) Levels of p62 mRNA in Neuro-2a cells were analyzed by Northern blotting. Values are expressed as means ± SEM of normalized pixel volumes (0 h = 1.0) from 3–4 separate experiments. $^{**}P < 0.01$, $^{*}P < 0.05$, compared to 0 h. (B) Protein expression of p62 in Neuro-2a cells was assessed by immunoblotting using p62-specific rabbit antiserum. (C) Neuro-2a cells were treated with PSI and ubiquitin immunoreactivity of whole-cell extracts at 0–36 h was analyzed by immunoblotting using a rabbit polyclonal antibody (C, untreated control, 36 h). (D, upper panels) Expression of p62 mRNA in rat hippocampal neurons treated with lactacystin, as determined by Northern blotting (C, untreated control, 24 h). As a loading control, 18S rRNA was stained with methylene blue. (Bottom panel) Protein level of p62 in rat hippocampal neurons treated with lactacystin was assessed by immunoblotting. (E) Rat hippocampal neurons were treated with lactacystin and ubiquitin immunoreactivity of whole-cell extracts was analyzed by immunoblotting.

creased the level of the 62-kDa protein at 24 h (Fig. 3C). The results demonstrate that the increase observed in p62 mRNA level is paralleled at the protein level, with similar time courses.

Proteasomal Inhibitors Induce a Prominent Upregulation of p62 mRNA and Protein in Neuro-2a Cells

The p62 protein has a ubiquitin-binding area in its N-terminal region and can bind ubiquitin noncovalently *in vitro* and *in vivo* (16), suggesting that it may play a regulatory role connected to the ubiquitin system. To address the question whether the mRNA and protein levels of p62 are affected by the accumulation of ubiquitinated proteins, we used the proteasomal inhibitors MG-132, lactacystin, and proteasome inhibitor I (PSI) to increase the level of ubiquitin-protein conjugates in Neuro-2a cells, and monitored the levels of p62 mRNA and protein (Figs. 4A–4C). Interestingly, the level of p62 mRNA was prominently upregulated by all three inhibitors tested (Fig. 4A), with varying time courses. In the case of MG-132, p62 mRNA reached a steady level as early as 6 h, whereas lactacystin caused a continuous increase of p62 mRNA during 0–24 h. The highest upregulation of p62 mRNA, over fourfold, was obtained with PSI.

At the protein level, p62 expression was similarly increased by all three inhibitors tested, with a maximum increase at 24–36 h (Fig. 4B). Interestingly, in the case of PSI, a higher M_w band appeared above the

62-kDa signal (Fig. 4B, bottom panel). Based on its apparent M_w at ~70 kDa, it is possible that this band represents p62 conjugated to monoubiquitin. The intensity of the upregulation of p62 expression in Neuro-2a cells by proteasomal inhibitors was similar to that caused by apoptotic treatments, with a two- to fourfold increase at the mRNA level (compare Fig. 4A to Fig. 2A), and a maximum increase at the protein level at 24–36 h (compare Fig. 4B to Figs. 3A and 3B).

In order to compare the expression profile of p62 to the level of ubiquitinated proteins, we examined the effect of proteasome inhibitors on the level of ubiquitin-immunoreactive proteins in Neuro-2a cells. As expected, proteasome inhibitor I (PSI) caused a pronounced increase in the level of ubiquitinated proteins during 12–36 h (Fig. 4C). Similar results were obtained using MG-132 and lactacystin (data not shown).

Proteasomal Inhibitors Induce Expression of p62 mRNA and Protein in Rat Hippocampal Neurons

Next we examined the effect of proteasomal inhibitors on p62 expression in primary cultured neurons (Figs. 4D and 4E). In rat hippocampal neurons treated with lactacystin, expression of p62 mRNA was prominently upregulated during 12–24 h (Fig. 4D, upper panels). Furthermore, the protein expression of p62 paralleled that of mRNA (Fig. 4D, bottom panel). As observed in Neuro-2a cells, lactacystin potently increased the amount of ubiquitin-immunoreactive proteins in rat hippocampal neurons (Fig. 4E).

Taken together, the results demonstrate, for the first time, that pro-apoptotic treatments and proteasomal inhibitors prominently increase the expression of p62 mRNA and protein in neuronal cells. In addition, the results suggest that the upregulation of p62 expression by proteasomal inhibitors could be a response to an increased level of ubiquitinated proteins.

DISCUSSION

In the present work, we report for the first time an association between neuronal apoptosis and upregulation of ubiquitin-binding protein p62 expression. From the viewpoint of neuropathology, several aspects of p62 warrant its study in the context of neuronal death: [i] rapid induction of p62 mRNA and protein by different stimuli, including oxidative stress (7, 17), [ii] ability to bind noncovalently to ubiquitin (16), [iii] involvement in protein aggregation (8, 9), [iv] binding to several intracellular signalling proteins, such as $p56^{lck}$, PKC-λ/ι, $p38^{MAPK}$, and RIP, some of which have been associated with cell stress or apoptosis (6, 18, 19), and [v] indirect evidence for a protective effect against excitotoxicity in rat brain (20). Oxidative stress and protein aggregation contribute to both chronic and acute neuropathological processes (11, 21). We therefore set out to explore the role of p62 under the conditions of neuronal apoptosis and proteasomal dysfunction *in vitro.*

The initial cDNA array observation that p62 mRNA is induced during serum deprivation of Neuro-2a cells prompted us to test the possibility that the induction of p62 is a common event in neuronal apoptosis. We observed that p62 mRNA was upregulated by serum deprivation, okadaic acid, etoposide, and trichostatin A at 12–24 h following the apoptotic treatment. Interestingly, the induction of p62 expression clearly preceded the activation of caspase-3, which appears at ~36 h in these models (13, 15). Therefore, p62 upregulation appears to occur well before the execution phase of apoptosis, temporally paralleling the activation of more upstream apoptotic signals. The response of p62 expression to diverse apoptotic treatments *in vitro* suggests a role for p62 also in neuronal apoptosis *in vivo.*

In Neuro-2a cells as well as in rat hippocampal neurons, the proteasomal inhibitors MG-132, lactacystin, and PSI prominently increased the expression of p62 protein and mRNA, with a concomitant accumulation of ubiquitinated proteins. The marked increase of p62 expression by proteasomal inhibitors suggests that the upregulation of p62 could be a response to an increased level of ubiquitinated proteins. Because of its ability to interact with ubiquitin as well as a number of signalling proteins, p62 is likely to have a regulatory role by forming a link between signal pathways and ubiquitin-mediated proteolysis. Ubiquitinated proteins accumulate in neurons in protein-damaging conditions, such as oxidative stress and excitotoxicity. Therefore, it is conceivable that by responding to increased levels of ubiquitinated proteins, p62 upregulation might have a protective effect in these pathological conditions.

A direct association of p62 with human disease was recently reported by demonstrating that p62 is a major constituent in intracytoplasmic protein aggregates (hyaline bodies) observed in hepatocellular carcinoma (9). The presence of p62 in protein aggregates *in vivo* and the induction of p62 expression in neuronal cells *in vitro* by protein aggregation-promoting conditions suggests that p62 may play a role also in neurodegenerative diseases, such as Alzheimer's disease (AD), since a hallmark of these diseases is the accumulation of intracellular protein aggregates in the brain.

Our results demonstrate a role for p62 during conditions of neuronal apoptosis and proteasomal dysfunction *in vitro.* It will be important to determine the significance of p62 during neuronal loss and the formation of protein aggregates in AD and other neuropathological conditions.

ACKNOWLEDGMENTS

The authors thank Dr. T. Ishii (Univ. of Tsukuba, Japan) for providing the A170 antiserum and M. Sc. Petri Kerokoski for critical reading of the manuscript.

REFERENCES

1. Snider, B. J., Gottron, F. J., and Choi, D. W. (1999) Apoptosis and necrosis in cerebrovascular disease. *Ann. NY Acad. Sci.* **893,** 243–253.
2. Stadelmann, C., Deckwerth, T. L., Srinivasan, A., Bancher, C., Bruck, W., Jellinger, K., and Lassmann, H. (1999) Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease. Evidence for apoptotic cell death. *Am. J. Pathol.* **155,** 1459–1466.
3. Anderson, A. J., Su, J. H., and Cotman, C. W. (1996) DNA damage and apoptosis in Alzheimer's disease: Colocalization with c-Jun immunoreactivity, relationship to brain area, and effect of postmortem delay. *J. Neurosci.* **16,** 1710–1719.
4. Villa, P., Miehe, M., Sensenbrenner, M., and Pettmann, B. (1994) Synthesis of specific proteins in trophic factor-deprived neurons undergoing apoptosis. *J. Neurochem.* **62,** 1468–1475.
5. Roschier, M., Kuusisto, E., and Salminen, A. (2000) A differential display protocol to identify differentially expressed mRNAs in potassium-deprived cerebellar granule cells. *Brain Res. Brain Res. Protoc.* **5,** 121–131.
6. Joung, I., Strominger, J. L., and Shin, J. (1996) Molecular cloning of a phosphotyrosine-independent ligand of the p56lck SH2 domain. *Proc. Natl. Acad. Sci. USA* **93,** 5991–5995.
7. Ishii, T., Yanagawa, T., Kawane, T., Yuki, K., Seita, J., Yoshida, H., and Bannai, S. (1996) Murine peritoneal macrophages induce a novel 60-kDa protein with structural similarity to a tyrosine kinase p56lck-associated protein in response to oxidative stress. *Biochem. Biophys. Res. Commun.* **226,** 456–460.
8. Puls, A., Schmidt, S., Grawe, F., and Stabel, S. (1997) Interaction of protein kinase C zeta with ZIP, a novel protein kinase C-binding protein. *Proc. Natl. Acad. Sci. USA* **94,** 6191–6196.
9. Stumptner, C., Heid, H., Fuchsbichler, A., Hauser, H., Mischinger, H. J., Zatloukal, K., and Denk, H. (1999) Analysis of intracytoplasmic hyaline bodies in a hepatocellular carcinoma. Demonstration of p62 as major constituent. *Am. J. Pathol.* **154,** 1701–1710.
10. Ciechanover, A. (1998) The ubiquitin-proteasome pathway: on protein death and cell life. *EMBO J.* **17,** 7151–7160.
11. Alves-Rodrigues, A., Gregori, L., and Figueiredo-Pereira, M. E. (1998) Ubiquitin, cellular inclusions and their role in neurodegeneration. *Trends Neurosci.* **21,** 516–520.
12. Brewer, G. J., Torricelli, J. R., Evege, E. K., and Price, P. J. (1993) Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. *J. Neurosci. Res.* **35,** 567–576.
13. Solovyan, V., Bezvenyuk, Z., Huotari, V., Tapiola, T., and Salminen, A. (1999) Distinct mechanisms underlay DNA disintegration during apoptosis induced by genotoxic and nongenotoxic agents in neuroblastoma cells. *Neurochem. Int.* **34,** 465–472.
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
15. Korhonen, P., Tapiola, T., Suuronen, T., and Salminen, A. (1998) Expression of transcriptional repressor protein mSin3A but not mSin3B is induced during neuronal apoptosis. *Biochem. Biophys. Res. Commun.* **252,** 274–277.
16. Vadlamudi, R. K., Joung, I., Strominger, J. L., and Shin, J. (1996) p62, a phosphotyrosine-independent ligand of the SH2 domain of p56lck, belongs to a new class of ubiquitin-binding proteins. *J. Biol. Chem.* **271,** 20235–20237.
17. Lee, Y. H., Ko, J., Joung, I., Kim, J. H., and Shin, J. (1998) Immediate early response of the p62 gene encoding a non-proteasomal multiubiquitin chain binding protein. *FEBS Lett.* **438,** 297–300.
18. Sanchez, P., De Carcer, G., Sandoval, I. V., Moscat, J., and Diaz-Meco, M. T. (1998) Localization of atypical protein kinase C isoforms into lysosome-targeted endosomes through interaction with p62. *Mol. Cell. Biol.* **18,** 3069–3080.
19. Sudo, T., Maruyama, M., and Osada, H. (2000) p62 functions as a p38 MAP kinase regulator. *Biochem. Biophys. Res. Commun.* **269,** 521–525.
20. Nakaso, K., Kitayama, M., Ishii, T., Bannai, S., Yanagawa, T., Kimura, K., Nakashima, K., Ohama, E., and Yamada, K. (1999) Effects of kainate-mediated excitotoxicity on the expression of rat counterparts of A170 and MSP23 stress proteins in the brain. *Brain Res. Mol. Brain Res.* **69,** 155–163.
21. Love, S. (1999) Oxidative stress in brain ischemia. *Brain Pathol.* **9,** 119–131.