Free Radical Research, 2002 Vol. 36 (11), pp. 1179–1184

Antioxidants Inhibit the Human Cortical Neuron Apoptosis Induced by Hydrogen Peroxide, Tumor Necrosis Factor Alpha, Dopamine and Beta-amyloid Peptide 1–42

SONIA MEDINA[a], MARCOS MARTÍNEZ[b] and ANGEL HERNANZ[a,*]

[a]*Department of Clinical Biochemistry, Hospital Universitario La Paz, Castellana 261, 28046, Madrid, Spain;* [b]*Department of Pathology, Hospital General, Castellón, Spain*

Accepted by Professor J. Vina

(Received 2 May 2002; In revised form 20 June 2002)

Several substances related to the neurodegenerative diseases of Alzheimer and Parkinson, such as hydrogen peroxide, tumor necrosis factor alpha, dopamine and beta-amyloid peptide 1–42, have been shown to induce apoptosis in tumoral cell lines and rat neurons but not in human neurons. Moreover, the role of mitochondria (membrane potential) during neuronal apoptosis is still a matter of debate. We present here, for the first time, in cultured human cortical neurons that the DNA fragmentation induced by these substances was preceded by a decrease of the mitochondrial membrane potential. We have also examined the antiapoptotic effect of the antioxidants glutathione, *N*-acetyl-cysteine and ascorbic acid. All these antioxidants inhibited the apoptosis induced by hydrogen peroxide, tumor necrosis factor alpha, dopamine and beta-amyloid peptide 1–42, since they were able to inhibit completely the mitochondrial membrane potential depolarization and the DNA fragmentation.

Keywords: Apoptosis; Antioxidants; Beta-amyloid peptides; Human cortical neurons; Neurodegenerative diseases

INTRODUCTION

Apoptosis is an important mechanism in both development and degeneration of the nervous system and also in pathophysiological states such as stroke, and glutamate and β-amyloid peptide toxicity. Evidence suggests that the loss of neurons in many neurologic disorders occurs by apoptosis.[1–4] It has been previously shown that exposure of certain cell types to low concentration of hydrogen peroxide (H_2O_2) can induce apoptosis, while higher concentrations invariably induce necrosis.[5]

Subjects with Alzheimer disease experience a progressive loss of cognitive function, resulting from a neurodegenerative process characterized classically by β-amyloid deposition in plaques and in the cerebral vasculature, as well as the formation of neurofibrillary tangles in neurons. It was shown that β-amyloid peptide induces apoptosis in mouse neuron cultures.[1] Su *et al.* (1994)[6] reported evidence for DNA fragmentation in neurons from subjects with Alzheimer's disease. Despite the high concentration of dopamine that exists in striatum, there is increasing evidence that dopamine, or one of its metabolites, might be neurotoxic because they can generate reactive oxidative species (ROS). The use of antioxidants has also provided an important tool for investigating the mechanism of dopamine toxicity. Thus, several investigators have demonstrated neuroprotection against dopamine-induced cell death by antioxidant administration in different neuron types.[7,8] Tumor necrosis factor alpha (TNFα) has also been well characterized as a mediator of oxidative stress and it produces apoptosis in non-neuronal[9] and neuronal cell lines.[10]

Experimental evidences recognize the mitochondria dysfunction as one of the important mediators

*Corresponding author. Fax: +34-917277090. E-mail: ahernanz@hulp.insalud.es

ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/10715762100006445

of apoptosis.[11,12] At least three mitochondrial specific events have been well defined in apoptosis, namely, loss of mitochondrial transmembrane potential, induction of mitochondrial permeability transition and cytosolic translocation of apoptogenic factors such as cytochrome C and apoptosis inducing factor.[13–16] In this context, H_2O_2 and β-amyloid peptide 1–40 has been shown to induce a decrease of mitochondrial membrane potential[17,18] in intact isolated nerve terminals and in a rat striatal cell line named M26-1F, respectively. However, the functionality of mitochondria during neuronal apoptosis is still a matter of debate. In fact, it has been postulated by several authors that mitochondrial depolarization is not required for neuronal apoptosis.[19–21]

ROS and the resulting oxidative stress play a key role in apoptosis. In some apoptosis models, alteration in the redox status of the cell to a more oxidizing environment occurs prior to activation of the final phase of caspase activation[12,22] and it has been proposed that this DNA fragmentation can be caused by oxidative damage.[23] This argument is further supported by the ability of several antioxidants such as *N*-aceytlcysteine (NAC) to block apoptosis.[24–26]

Thus, the aim of the present work was to study the apoptosis of human cortical neurons and the role of some antioxidants as antiapoptotic factors induced by several substances such as β-amyloid peptide 1–42, TNFα, dopamine and H_2O_2 implicated in neurodegenerative diseases. Similar studies have been carried out with human or animal tumoral cell lines and non-human neurons, however under our knowledge no such studies have been performed with human cortical neurons.

MATERIAL AND METHODS

Cortical Neuron Cultures

The commercial human HCN-1A cell line (ATCC, Manassas VA) was derived from cortical tissue removed from a patient undergoing hemispherectomy for intractable seizures. Cortical neurons were plated in 96-well plates at 5×10^4 cells/well and incubated in Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL, Life Technologies) containing 100 ng/ml of nerve growth factor (NGF 7S, Roche, Mannheim, Germany), 250 U/ml penicillin and 100 μg/ml streptomycin (Roche Diagnostic, Mannheim, Germany). All of the experiments were performed in serum-free DMEM to avoid the disturbing effect of antioxidants that the fetal bovine serum contains. Cultures were incubated at 37°C in a humidified atmosphere composed of 5% CO_2.

Induction of Apoptosis

Cells were exposed to 10 μM H_2O_2 (Merck, Dannstad, Germany), 200 ng/ml of TNFα, 50 μM of dopamine or 10 μM of β-amyloid peptide 1–42 (Sigma, St Louis, MO). The β-amyloid peptide was pretreated at 37°C during seven days. The different substances were diluted in phosphate-buffered saline solution (PBS). The antioxidants, probed to revert the apoptosis induced by the different substances above mentioned, were *N*-acetyl-cysteine (5 mM), glutathione (5 mM) or ascorbic acid (100 μM), all of them obtained from Sigma (St Louis, MO). The different apoptotic inductors were added to the wells containing the cortical neurons alone or together with the different antioxidants.

Estimation of Mitochondrial Membrane Potential

JC-1 (Molecular Probes, Eugene, OR) is a lipophilic cation that is able to selectively enter into mitochondria and it allows an estimation of membrane potential. JC-1 exists in a monomeric form emitting at 535 nm after excitation at 485 nm when the mitochondrial membrane potential decreases. Stock solution of JC-1 was prepared in dimethylsulfoxide (DMSO), and the concentration employed in the assay was obtained by PBS dilution. Cell suspensions were adjusted to a density of 5×10^4 cells/well and incubated in DMEM medium with JC-1 (10 μg/ml) at 37°C in the dark. At 30 min of the incubation period the cells were washed in PBS, resuspended in a total volume of 200 μl and the intensity of fluorescence was analyzed in a multiwell fluorescence scanner (Wallac 1420 multilabel counter, Perkin Elmer).

Assessment of Apoptosis

To observe nuclear DNA fragmentation occurring during apoptosis the chromatin-specific dye Hoechst 33342 (Molecular Probes, Eugene, OR) was used. Bisbenzimides are cell-permeant, adenine–thymine binding fluorescent dyes used to evaluate DNA fragmentation. Hoechst 33342 has been defined as the best probe for identifying early apoptosis cells by their characteristic nuclear condensation and fragmentation in flow cytometry studies.[27] Cells were stained after different times of incubation (from 2 to 6 h) with 1.5 μg/ml Hoechst 33342. The degree of DNA fragmentation induced by the different substances studied was measured by a multiwell fluorescence detector.

Neuron Viability

At the final period of incubation (6 h) with the different inductors and antioxidants, cells were

stained with trypan blue, washed with PBS and fixed to the 96-well plate in order to measure neuron viability. Apoptotic cells were distinguished from those living morphologically by rounded, condensed nuclei, in contrast to larger, oval, healthy nuclei, and the presence of membrane blebbling in the apoptotic cells. Necrotic cells were stained with trypan blue whereas apoptotic and living cells did not.

Statistics

Data are given as mean ± SD of three experiments performed in duplicate. Comparisons between groups were assessed with *U*-Mann–Whitney and Friedman test for nonparametric samples. The p values smaller than 0.05 were considered to be statistically significant.

RESULTS

We have shown in this study that human cortical neurons in culture are susceptible to apoptosis induced by H_2O_2, β-amyloid 1–42, dopamine and TNFα. It can be observed in Fig. 1 that these compounds induced a statistically significant depolarization of the mitochondrial membrane potential at 30 min, which is measured by an increase in the fluorescence at 535 nm of the monomeric form of the dye JC-1. When the different antioxidants used, NAC, glutathione or ascorbic acid, were added together with the different apoptosis inductors, the mitochondrial membrane depolarization was completely inhibited.

The apoptotic compounds (H_2O_2, β-amyloid 1–42, dopamine and TNFα) induced a statistically significant increase of DNA fragmentation (Fig. 2), also inhibited by the three antioxidants. In Fig. 3, it can be observed the time course of DNA fragmentation after the different apoptotic substances were added. A maximum rate of DNA fragmentation, between 4 and 5 h, was observed. Antioxidants did not produce changes respect to the control values when they were added alone to neuron cultures. In Fig. 3, we have only represented NAC values.

After the final period of incubation (6 h) the percentage of cell viability was measured using the method of trypan blue exclusion by counting by microscope the living cells. Thus, Fig. 4 shows a statistically significant decrease of the cell viability when dopamine, H_2O_2, TNFα or β-amyloid peptide 1–42 were added to the cortical neuron cultures. The decrement in the cell viability was totally reverted by the three antioxidants used. When the antioxidants were added alone to the human cortical neurons, they induced a slight increment in the viability of these cells.

DISCUSSION

In the present study, we have evaluated in human cortical neurons two key apoptotic events produced by different substances related to the neurodegenerative diseases Alzheimer and Parkinson. Thus, we have found that β-amyloid peptide 1–42, TNFα, H_2O_2 and dopamine induce apoptosis associated with mitochondrial membrane potential

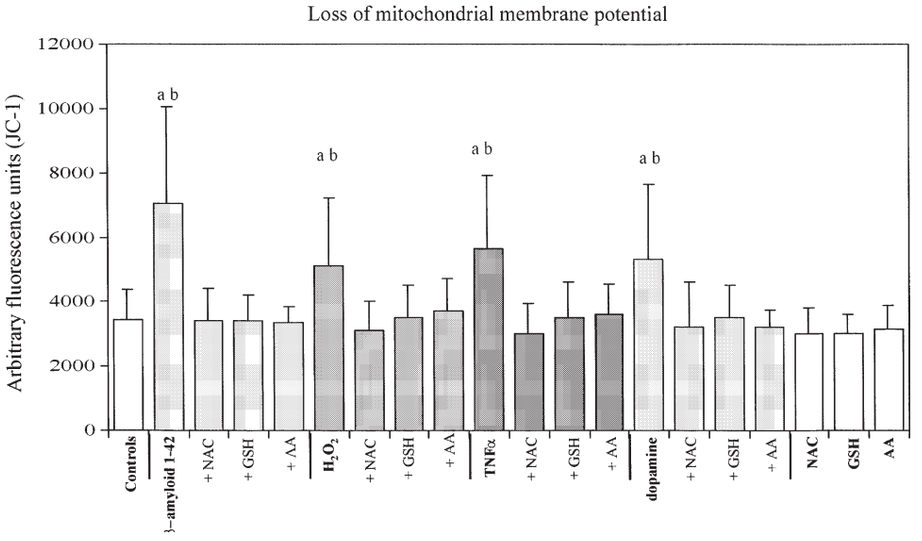

FIGURE 1 Changes at 30 min in the mitochondrial membrane potential induced by β-amyloid peptide 1–42, hydrogen peroxide (H_2O_2), tumor necrosis factor alfa (TNFα) and dopamine on human cortical neuron cultures measured as monomer JC-1 fluorescence, with and without the presence of *N*-acetyl-cysteine (NAC), glutathione (GSH) and ascorbic acid (AA). The results represent the mean ± SD of three values corresponding to three experiments, each value being the mean of duplicate assays. (a) $p < 0.01$ with respect to control values; (b) $p < 0.01$ with respect to antioxidant addition.

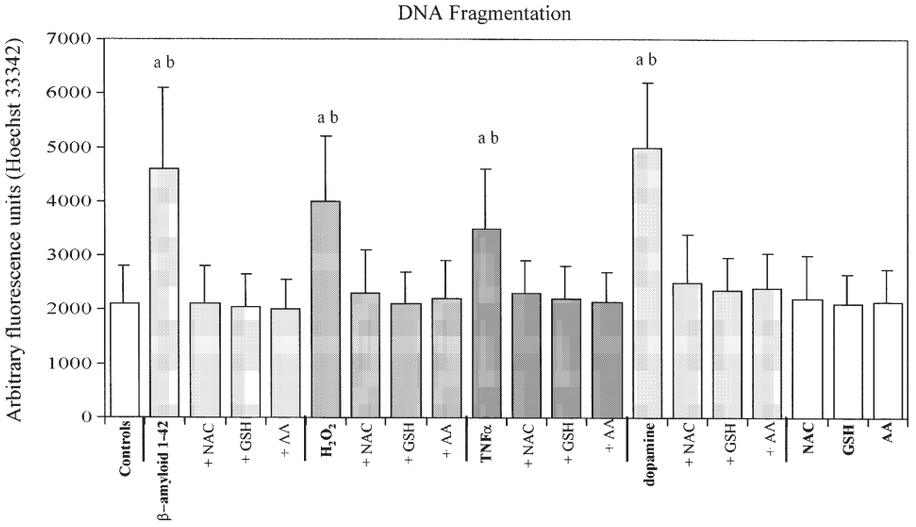

FIGURE 2 DNA fragmentation at 6 h induced by β-amyloid peptide 1–42, hydrogen peroxide (H_2O_2), tumor necrosis factor alfa (TNFα) and dopamine on human cortical neuron cultures measured as Hoechst 33342 fluorescence, with and without the presence of *N*-acetyl-cysteine (NAC), glutathione (GSH) and ascorbic acid (AA). The results represent the mean ± SD of three values corresponding to three experiments, each value being the mean of duplicate assays. (a) $p < 0.01$ with respect to control values; (b) $p < 0.01$ with respect to antioxidant addition.

depolarization. ROS seems to play a pivotal role in neuronal apoptosis since it can be blocked by antioxidants. Thus, we have found that NAC, glutathione and ascorbic acid prevented the apoptosis induced by the different apoptotic substances mentioned above. To our knowledge, similar studies have been performed in rat or mouse neurons or tumoral cell lines derived from rat or human origin, but no studies have been performed with human cortical neurons.

Since recent studies present opposite results about the existence of a decrease of the mitochondrial membrane potential before the neuronal apoptosis,[12,13,19–21,28] we asked the question whether the mitochondrial membrane potential depolarization was involved in apoptosis of human cortical neurons. Thus, we have found that membrane depolarization seems to be an initial step that occurs in the neuronal apoptosis induced by H_2O_2, β-amyloid peptide 1–42, TNFα or dopamine.

It has been demonstrated that ROS are required for TNFα mediated neuronal apoptosis, since cell death can be blocked by the antioxidant NAC. NAC at a dose of 0.5 mM completely blocked TNFα-mediated

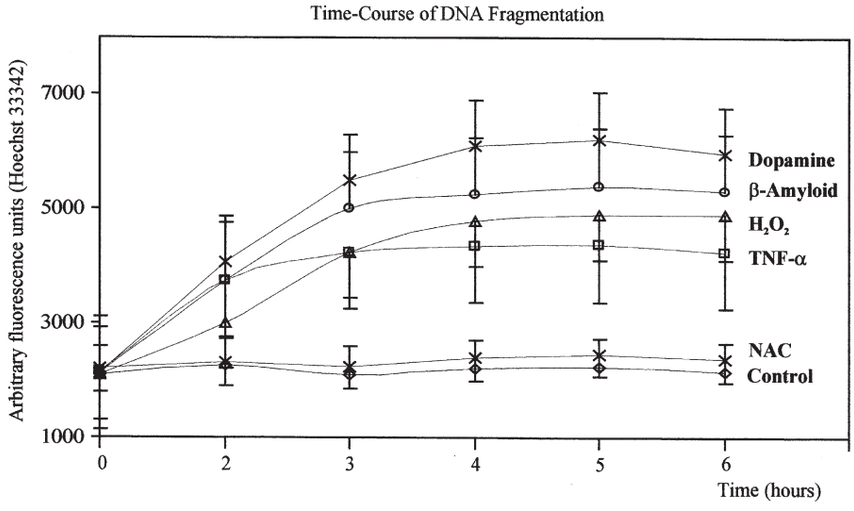

FIGURE 3 Time course of the DNA fragmentation induced by β-amyloid peptide 1–42, hydrogen peroxide (H_2O_2), tumor necrosis factor alfa (TNFα) and dopamine on human cortical neuron cultures, represented as arbitrary units of Hoechst 33342 fluorescence. The results represent the mean ± SD of three values corresponding to three experiments, each value being the mean of duplicate assays.

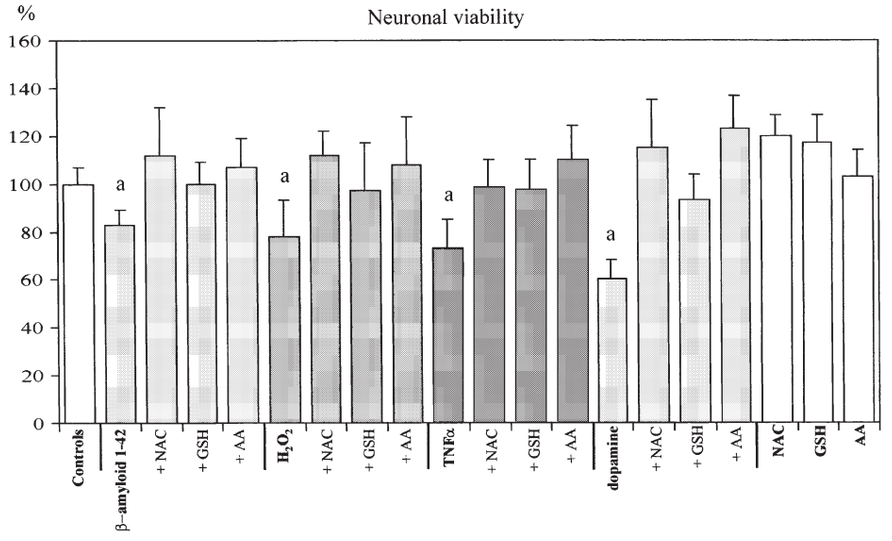

FIGURE 4 Neuron viability at the final period of incubation (6 h) measured by the trypan blue exclusion test. Human neurons were exposed to β-amyloid peptide 1–42, hydrogen peroxide (H_2O_2), tumor necrosis factor alfa (TNFα), with and without the presence of N-acetyl-cysteine (NAC), glutathione (GSH) and ascorbic acid (AA). The results represent the mean ± SD of three values corresponding to three experiments, each value being the mean of duplicate assays. (a) $p < 0.01$ with respect to control values.

cytotoxicity in neuronal SK-N-MC cells cultures.[10] Ascorbic acid at its maximal non-toxic dose[29] did not seem as effective as 1 mM NAC. In the present work, the apoptotic effect exerted by TNFα was abolished by the three antioxidants studied, NAC, ascorbic acid and glutathione. The same NAC concentration (5 mm) employed in the present work has been described to do cells less susceptible to TNFα-mediated injury in a myelomonocytic human cell line.[30]

There is an evidence suggesting that oxidative stress also plays a key role in the β-amyloid-mediated neurotoxicity because increased levels of H_2O_2 were detected.[31,32] Antioxidants such as vitamin E, estrogens or melatonin have demonstrated neuroprotective effects on β-amyloid peptide-mediated cytotoxicity.[33–35] Our data are in agreement with this fact, since the three antioxidants studied were able to abolish the apoptotic effect created not only by β-amyloid peptide 1–42 but also by the other apoptosis inductors. In the present study, β-amyloid peptide 1–42 at 10 μM concentration induced neuronal apoptosis in a short time period. Thus, a 30-min period was sufficient to induce membrane mitochondrial depolarization, whereas the DNA fragmentation was found between 3 and 4 h of incubation. Similar studies performed with cortical cultures from mouse embryos exposed to 25 μM amyloid peptide showed neuronal apoptosis, appearing within 4–6 h of amyloid peptide treatment.[1]

In human melanoma M14 cell line of 0.5 mM H_2O_2 induced apoptosis whereas 1–2 mM concentrations did necrosis.[5] Moreover, rat thymocyte suspensions treated with 100 μM H_2O_2 died mainly via apoptosis whereas at concentrations higher than 100 μM necrosis was predominant.[36] In our study, a concentration of 10 μM H_2O_2 was sufficient enough to induce apoptosis in human cortical neurons, and therefore it is possible that human cortical neurons can be more sensitive to the H_2O_2 exposure. In PC12 cells, NAC or ascorbic acid inhibited the apoptosis/necrosis induced by higher concentrations of dopamine (100–500 μM)[7,8] than those employed in the present study (50 μM). We have observed in this work that neuronal apoptosis induced by dopamine was reverted when antioxidants were added. Thus, dopamine induces probably this apoptosis mediated by oxidative stress.

Antioxidants also act in the maintenance of mitochondrial homeostasis and has been demonstrated in the present work. Given the fact that the three antioxidants studied seems to play an important antiapoptotic action against different apoptotic inductors used, it is possible that oxidative stress plays a relevant role in the neuronal apoptosis of human cortical neurons, and it would be a common fact to begin apoptosis produced by β-amyloid peptide, dopamine, TNFα and H_2O_2. If these findings in neuron cultures are confirmed *in vivo*, then the neuroprotective effects of antioxidants may provide an important therapeutic approach in the prevention of several neurodegenerative diseases such as Alzheimer and Parkinson.

Acknowledgements

This work has been supported by a FIS grant (0334/98) from the Ministerio de Sanidad y Consumo of Spain.

References

[1] Loo, D.T., Copani, A., Pike, C.J., Whittemore, E.R., Walencewicz, A.J. and Cotman, C.W. (1993) "Apoptosis is induced by beta-amyloid in cultured central nervous system neurons", *Proc. Natl Acad. Sci. USA* **90**, 7951–7955.

[2] Portera-Cailliau, C., Hedreen, J.C., Price, D.L. and Koliatsos, V.E. (1995) "Evidence for apoptotic cell death in Huntington disease and excitotoxic animal models", *J. Neurosci.* **15**, 3775–3787.

[3] Lipton, S.A. (1996) "Similarity of neural cell injury and death in AIDS dementia and focal cerebral ischemia: potential treatment with NMDA open-channel blockers and nitric oxide-related species", *Brain Pathol.* **6**, 507–517.

[4] Burke, R.E. and Kholodilov, N.G. (1998) "Programmed cell death: does it play a role in Parkinson's disease?", *Ann. Neurol.* **44**, 126–133.

[5] Clément, M.V., Ponton, A. and Pervaiz, S. (1998) "Apoptosis induced by hydrogen peroxide is mediated by decreased superoxide anion concentrations and reduction of intracellular milieu", *FEBS Lett.* **440**, 13–18.

[6] Su, J.H., Anderson, A.J., Cummings, B.J. and Cotman, C.W. (1994) "Immunochemical evidende for apoptosis in Alzheimer's disease", *Neuroreport* **5**, 2529–2533.

[7] Jones, D.C., Gunasekar, P.G., Borowith, J.L. and Isom, G.E. (2000) "Dopamine-induced apoptosis is mediated by oxidative stress and is enhanced by cyanide in differentiated PC12 cells", *J. Neurochem.* **74**, 2296–2304.

[8] Offen, D., Ziv, I., Sternin, H., Melamed, E. and Hochman, A. (1996) "Prevention of dopamine-induced cell death by thiol antioxidants: possible implications for treatment of Parkinson's disease", *Exp. Neurol.* **141**, 32–39.

[9] Larrick, J.W. and Wright, S.C. (1990) "Cytotoxic mechanism of tumor necrosis factor-alpha", *FASEB J.* **4**, 3215–3223.

[10] Talley, A.K., Dewhurst, S., Perry, S.W., Dollard, S.C., Gummuluru, S., Fine, S.M., New, D., Epstein, L.G., Gendelman, H.E. and Gelbard, H.A. (1995) "Tumor necrosis factor alpha-induced apoptosis in human neuronal cells: protection by the antioxidant *N*-acetylcysteine and the genes bcl-2 and crmA", *Mol. Cell. Biol.* **15**, 2359–2366.

[11] Zamzami, N., Susin, S.A., Marchetti, P., Hirsch, T., Gomez Monterrey, I., Castedo, M. and Kroemer, G. (1996) "Mitochondrial control of nuclear apoptosis", *J. Exp. Med.* **183**, 1533–1544.

[12] Susin, S.A., Zamzami, N. and Kroemer, G. (1998) "Mitochondria as regulators of apoptosis; doubt no more", *Biochim. Biophys. Acta* **1366**, 151–165.

[13] Bossy-Wetzel, E., Newmeyer, D.D. and Green, D.R. (1998) "Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization", *EMBO J.* **17**, 37–49.

[14] Fulda, S., Scaffidi, C., Susin, S.A., Krammer, P.H., Kroemer, G., Peter, M.E. and Debatin, K.M. (1998) "Activation of mitochondria and release of mitochondrial apoptogenic factors by betulinic acid", *J. Biol. Chem.* **273**, 33942–33948.

[15] Troyan, M.B., Gilman, V.R. and Gay, C.V. (1997) "Mitochondrial membrane potential changes in osteoblasts treated with parathyroid hormone and estradiol", *Exp. Cell Res.* **233**, 247–280.

[16] Zamzami, N., Marchetti, P., Castedo, M., Zanin, C., Vayssiere, J.L., Petit, P.X. and Kroemer, G. (1995) "Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death *in vivo*", *J. Exp. Med.* **181**, 1661–1672.

[17] Chinopoulos, C., Tretter, L. and Adam-Vizi, V. (1999) "Depolarization of *in situ* mitochondria due to hydrogen peroxide-induced oxidative stress in nerve terminals: inhibition of alpha-ketoglutarate dehydrogenase", *J. Neurochem.* **73**, 220–228.

[18] Laskay, G., Zarandi, M., Jost, K., Penke, B., Balint, E., Oscouszki, I., Tarcsa, M., Varszegi, S. and Gulya, K. (1999) "Beta-amyloid (1,40)-induced early hyperpolarization in M26-1F cells, an immortalized rat striatal cell line", *Neurobiology* **7**, 431–436.

[19] Krohn, A.J., Wahlbrink, T. and Prehn, J.H.M. (1999) "Mitochondrial depolarization is not required for neuronal apoptosis", *J. Neurosci.* **19**, 7394–7404.

[20] Budd, S.L., Tenneti, L., Lishnak, T. and Lipton, S.A. (2000) "Mitochondrial and extramitochondrial apoptotic signaling pathways in cerebrocortical neurons", *PNAS* **97**, 6161–6166.

[21] Jouvet, P., Rustin, P., Taylor, D.L., Pocock, J.M., Felderhoff-Mueser, U., Mazarakis, N.D., Sarraf, C., Joashi, U., Kozma, M., Greenwood, K., Edwards, A.D. and Mehmet, H. (2000) "Branched chain amino acids induce apoptosis in neural cells without mitochondrial membrane depolarization or cytochrome c release: implications for neurological impairment associated with Maple Syrup urine disease", *Mol. Biol. Cell* **11**, 1919–1932.

[22] Green, D.R. and Reed, J.C. (1988) "Mitochondria and apoptosis", *Science* **281**, 1309–1312.

[23] Tsang, S.Y., Tam, S.C., Bremner, I. and Burkitt, M.J. (1996) "Research communication copper-1, 10-phenanthroline induces internucleosomal DNA fragmentation in HepG2 cells, resulting from direct oxidation by the hydroxyl radical", *Biochem. J.* **317**, 13–16.

[24] McGowan, A.J., Fernandes, R.S., Samali, A. and Cotter, T.G. (1996) "Anti-oxidants and apoptosis", *Biochem. Soc. Trans.* **24**, 229–233.

[25] Hockenbery, D.M., Oltvai, Z.N., Yin, X.M., Milliman, C.L. and Korsmeyer, S.J. (1993) "Bcl-2 functions in an antioxidant pathway to prevent apoptosis", *Cell* **75**, 241–251.

[26] Ferrari, G., Yan, C.Y. and Greene, L.A. (1995) "*N*-acetylcysteine (D- and L-steroisomers) prevents apoptotic death of neuronal cells", *J. Neurosci.* **15**, 2857–2866.

[27] Elstein, K.H. and Zucker, R.M. (1994) "Comparison of cellular and nuclear flow cytometric techniques for discriminating apoptotic subpopulations", *Exp. Cell Res.* **211**, 322–331.

[28] Heiskanen, K.M., Bhat, M.B., Wang, H.W., Ma, J. and Nieminen, A.L. (1999) "Mitochondrial depolarization accompanies cytochrome c release during apoptosis in PC6 cells", *J. Biol. Chem.* **274**, 5654–5658.

[29] Mayer, M. and Noble, M. (1994) "*N*-acetyl-L-cysteine is a pluripotent protector against cell death and enhancer of trophic factor-mediated cell survival *in vitro*", *Proc. Natl Acad. Sci. USA* **91**, 7496–7500.

[30] Cossarizza, A., Franceschi, C., Monti, D., Salvioli, S., Bellesia, E., Rivabene, R., Biondo, L., Rainaldi, G., Tinari, A. and Malorni, W. (1995) "Protective effect of *N*-acetylcysteine in tumor necrosis factor-alpha-induced apoptosis in U937 cells: the role of mitochondria", *Exp. Cell Res.* **220**, 232–240.

[31] Behl, C., Davis, J.B., Lesley, R. and Schubert, D. (1994) "Hydrogen peroxide mediates amyloid beta protein toxicity", *Cell* **77**, 817–827.

[32] Schubert, D., Behl, C., Lesley, R., Brack, A., Dargusch, R., Sagara, Y. and Kimura, H. (1995) "Amyloid peptides are toxic via a common oxicative mechanism", *Proc. Natl Acad. Sci. USA* **92**, 1989–1993.

[33] Behl, C., Davis, J.B., Cole, G.M. and Schubert, D. (1992) "Vitamin E protects nerve cells from amyloid beta protein toxicity", *Biochem. Biophys. Res. Commun.* **186**, 944–950.

[34] Pappolla, M.A., Sos, M., Omar, R.A., Bick, R.J., Hickson-Bick, D.L., Reiter, R.J., Efthimiopoulos, S. and Robakis, N.K. (1997) "Melatonin prevents death of neuroblastoma cells exposed to the Alzheimer amyloid peptide", *J. Neurosci.* **17**, 1683–1690.

[35] Bonnefont, A.B., Muñoz, F.J. and Inestrosa, N.C. (1998) "Estrogen protects neuronal cells from the cytotoxicity induced by acetylcholinesterase-amyloid complexes", *FEBS Lett.* **441**, 220–224.

[36] Sakamoto, T., Repasky, W.T., Uchida, K., Hirata, A. and Hirata, F. (1996) "Modulation of cell death pathways to apoptosis and necrosis of H_2O_2-treated rat thymocytes by lipocortin I", *Biochem. Biophys. Res. Commun.* **220**, 643–664.