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ORIGINAL ARTICLE

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C-Phycocyanin protects cerebellar granule cells from low potassium/serum deprivation-induced apoptosis

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Abstract We tested the potential cytoprotective role of C-phycocyanin in rat cerebellar granule cell cultures. Cell death was induced by potassium and serum (K/S) withdrawal. Cell viability was studied using the neutral red assay and laser scanning cytometry with propidium iodide as fluorochrome.

C-phycocyanin (1–3 mg/ml) showed a neuroprotective effect against 24 h of K/S deprivation in cerebellar granule cells. After 4 h K/S deprivation this compound (3 mg/ml) inhibited formation of reactive oxygen species, measured as 2′,7′-dichlorofluorescein fluorescence, showing its scavenger capability. Pre-treatment with C-phycocyanin reduced thymidine incorporation into DNA below control values and reduced dramatically apoptotic bodies as visualized by propidium iodide, indicating inhibition of apoptosis induced by K/S deprivation.

Flow cytometry studies, using propidium iodide in TritonX100 permeabilized cells, indicated that 24 h K/S deprivation acts as a proliferative signal for cerebellar granule cells, which show an increase in S-phase percentage and cells progressed into the apoptotic pathway. C-phycocyanin protected cerebellar granule cells from the apoptosis induced by deprivation. These results suggest that C-phycocyanin prevents apoptosis in cerebellar granule cells probably through the antioxidant activity. It is proposed that K/S deprivation-induced apoptosis could be due, in part, to an alteration in the cell cycle mediated by an oxidative stress mechanism.

Key words C-phycocyanin · Oxidative stress · Cell cycle · Apoptosis · Laser scanning cytometry · Flow cytometry · Cerebellar granule cells · Rat

Introduction

Oxidative damage is thought to be important in neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases (Coyle and Puttfarcken 1993; Simonian and Coyle 1996; Pettmann and Henderson 1998; Reiter 1998; Skaper et al. 1998). Reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and hydroxyl radicals, are produced in neurons under normal conditions but when the intracellular levels of ROS are increased neuronal cell death occurs (Greenlund et al. 1996; Aoshima et al. 1997). The vulnerability of neurons to oxidative damage may be associated with a high rate of oxygen consumption, high levels of polyunsaturated fatty acids and also the presence of iron, a metal involved in the generation of ROS (Facchinetti et al. 1998; Tan et al. 1998).

Neurons are particularly vulnerable to free radical-induced damage because they contain low levels of antioxidants and detoxifying enzyme activities, such as catalase or superoxide dismutase, are reduced (Simonian and Coyle 1996; Raha and Robinson 2000) and glutathione levels also are low (Mattson 1998; Kannan and Jain 2000). Moreover, neurons are non-replicating cells and alterations in cellular processes may induce irreversible dysfunction (damage) in the central nervous system (CNS). Therefore, agents that prevent ROS formation are expected to exert protective actions in neurons subjected to oxidative stress (Busciglio and Yankner 1995; Sun and Chen 1998; Sastry and Rao 2000).

In recent years, several antioxidant drugs have been examined for their ability to prevent ROS-induced neuronal cell death, namely lazaroids (Camins et al. 1998),

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vitamin E (Schulz et al. 1996), melatonin (Reiter 1998) and carnosine (Boldyrev et al. 1999). The search for agents with antioxidant neuroprotective action is, therefore, an important issue in neurological research. Recently, we have demonstrated that C-phycocyanin, a biliprotein pigment found in some blue-green algae *Spirulina* (*Arthospira* sp.) (MacColl and Guard-Friar 1987), prevents the neurotoxic effects of kainic acid in rats (Rimbau et al. 1999). Other reports have indicated that C-phycocyanin has anti-inflammatory (Romay et al. 1998a, 1999) and hepatoprotective effects (Vadiraja et al. 1998). Evidence is accumulating that C-phycocyanin may function as a highly potent hydroxyl and peroxyl free radical scavenger and its therapeutic effects are due probably to this antioxidant activity (Romay et al. 1998a, 1998b, 1999; Lissi et al. 2000).

Little is known about the effects of C-phycocyanin in neuronal cell cultures. Cerebellar granule cells (CGC) are a widely used model in neuroscience due to their homogeneity and suitability for the study of molecular mechanisms of apoptosis (Araki et al. 1998). Dissociated CGC from early postnatal rats can be maintained in serum-containing medium by elevating the extracellular [K^+] (25 mM) (Skaper et al. 1998; Toescu 1998). Removal of serum and reducing [KCl] to 5 mM (K/S deprivation) triggers a cell death mechanism that is morphologically apoptotic and is accompanied by DNA fragmentation (Oka et al. 1996; Skaper et al. 1998; Vitolok et al. 1998; Padmanabhan et al. 1999). The biochemical events associated with K/S deprivation-induced apoptosis in CGC have been characterized. This apoptotic process is associated with an early increase in the levels of intracellular Ca^{2+}, increased ROS production and decreased ATP levels (Atabay et al. 1996; Galli et al. 1995; Berry 1999; Canu et al. 1999).

ROS are essential mediators of K/S deprivation-induced apoptosis in CGC (Schulz et al. 1996). In the present study we report a putative function of this natural pigment in K/S withdrawal-induced apoptosis of CGC, based on the proposed free radical scavenger activity of C-phycocyanin. We show the antiapoptotic properties of C-phycocyanin, accompanied by a marked reduction of ROS in accordance with its free-radical scavenging characteristics, and neuroprotective activity in CGC.

Materials and methods

Chemicals

Propidium iodide (PI), neutral red, DNAase, RNAase and 2′,7′-dichlorofluorescein (DCF) diacetate were purchased from Sigma (St. Louis, Mo., USA). c-Arabinoside, glutamine, gentamycin and KCl were tissue culture reagents purchased from Sigma. Mowiol 4–88 was from Calbiochem (Darmstadt, Germany). C-phycocyanin was obtained from *Arthospira maxima* sp. and purified using the method of Neufeld and Riggs (1969).

Cell culture

Primary cultures of CGC were prepared from 7-day-old Sprague Dawley rat pups according to the method of Nicoletti et al. (1986). Cerebella freed of meninges were trypsinized and treated with DNAase. Cell concentration was adjusted to 8×10^5 cells/ml and the cells plated on poly-L-lysine-coated 24-well plates (Falcon) at a density of 320,000 cells/cm^2. Cultures were grown in Eagle's medium (Eagle's basal medium, BME, Gibco, Life Technologies, Paisley, UK) containing 10% FCS (Gibco), 2 mM L-glutamine, 0.1 mg/ml gentamycin and 25 mM KCl. Cytosine arabinoside (10 μM) was added 16–18 h after plating to inhibit the growth of non-neuronal cells. Cultures prepared by this method were enriched in granule neurons more than 95%.

Treatment of CGC

After 6–7 days in vitro, CGC were washed and incubated for 24 h in complete medium containing C-phycocyanin (1–3 mg/ml). Thereafter the complete medium was replaced by medium containing C-phycocyanin but lacking serum and with [KCl] reduced to 5 mM (K/S-deprived medium). Survival was assessed 24 h after K/S deprivation employing both the neutral red assay and PI staining using the laser scanning cytometer (LSC).

Cell survival and detection of apoptosis

Neutral red cell viability assay

After 24 h K/S deprivation, the medium was removed and replaced with fresh complete medium containing 40 μg/ml neutral red (Babich and Borenfreund 1991). If necessary, the neutral red solution (5 mg/ml) was pre-incubated overnight at 37 °C and centrifuged (1,500 *g* for 10 min) prior to use to remove the fine precipitate of dye crystals. The assay plate was then returned to the incubator for another 3 h to allow for uptake of the supravital dye into viable CGC. Thereafter, the media was removed and the cells were rapidly washed twice with 1 ml PBS followed by 0.5 ml of a solution of 1% acetic acid-50% ethanol to extract the dye from the cells. After 10 min at room temperature 0.2 ml extract was transferred to a microplate spectrophotometer equipped with a 540-nm filter to measure the absorbance of the extracted dye. The absorbance of control wells was assumed to represent 100% viability.

Viability measured by LSC

Cell viability was measured using an LSC (CompuCyte, Cambridge, Mass., USA) equipped with a krypton/argon laser operating at 488 nm and 568 nm. CGC were grown on poly-L-lysine-coated glass cover-slips and treated as described above with C-phycocyanin (3 mg/ml) with complete or K/S-deprived medium. To assess the fluorescence intensity of the cells, the cover-slips were incubated for at least 10 min with PI (10 μg/ml), mounted on a glass slide and placed on the LSC microscope stage. In all experiments a single red fluorescence detector (590 nm) was used and the fluorescence of 3,000 CGC in each experiment was measured.

Cell and nuclear morphology. Apoptotic features after K/S deprivation

CGC were grown on cover-slips as described above. For cell morphology, neurons were observed using phase-contrast microscopy and representative images were captured with a Leica DMRB fluomicroscope using the 20× objective.

PI staining was used to obtain morphological evidence of apoptosis. CGC were fixed in cold 70% methanol for 20 min. After washing with PBS they were incubated for 3 min with a solution of PI in PBS (10 μg/ml). After washing, cover-slips were mounted in Mowiol 4–88. Stained cells were visualized under UV illumination (Leica DMRB fluomicroscope) and their digitized images captured.

Analysis of apoptosis rate by flow cytometry

Apoptosis was assessed after 24 h K/S deprivation. The culture medium was removed and the cells washed with PBS and collected from the culture plates by pipetting. The cell pellet was fixed in 70% ethanol at 4 °C for 2 h. After centrifugation at 100 *g* for 2 min, cells were resuspended in a solution of ethanol containing 150 µg/ml RNAase and incubated for 30 min at room temperature. Cells were then pelleted by centrifugation and resuspended in PBS. PI (10 µg/ml) was added 30 min before cytofluorometry analysis. Flow cytometer experiments were carried out using a flow cytometer (Epics XL, Coulter Corporation, Hialeah, Fla., USA). The instrument was set up with the standard configuration: sample excitation using as a standard 488 nm air-cooled argon-ion laser at 15 mW. Forward scatter (FSC), side scatter (SSC) and red (620 nm) fluorescence for PI were acquired. Optical alignment was based on optimized signal from 10-nm fluorescent beads (Immunocheck, Epics Division). Time was used as a control of the stability of the instrument. Red fluorescence was projected onto a 1024-monoparametrical histogram. Aggregates were excluded, gating single cells by their area vs. peak fluorescence signal. DNA analysis (Ploidy analysis) on single fluorescence histograms was done using Multicycle software (Phoenix Flow Systems, San Diego, Calif., USA).

[^{3}H]Methyl-thymidine incorporation assay

DNA synthesis was assessed by measuring [^{3}H]methyl-thymidine incorporation. Briefly, CGC were labelled for 20 h with 1 µCi [^{3}H]methyl-thymidine (specific activity 25 Ci/mmol, Amersham) in complete (control) or K/S-deprived medium, in the presence or absence of C-phycocyanin. At the end of the incubation, cultures were washed twice with ice-cold PBS and incubated for 30 min in 1 M ice-cold $HClO_4$. After two additional washings with 0.5 M $HClO_4$ and a final wash with ethanol, precipitates were solubilized with 0.5 M NaOH and neutralized with 0.5 M HCl. [^{3}H]Methyl-thymidine incorporation was measured by scintillation counting. Results are expressed as the percentage with respect to the maximal response with complete medium. Experiments were performed in quadruplicate at least three times.

Assessment of ROS production

ROS production was measured after 4 h K/S deprivation (Schulz et al. 1996), using DCF diacetate as previously described (Sureda et al. 1998) with minor modifications. DCF diacetate diffuses across the neuronal membranes and is trapped within the neurons by deacetylation. Deacetylated DCF can be oxidized by intracellular ROS to generate fluorescent DCF. The intensity of fluorescence (excitation at 488 nm, emission at 510 nm) is proportional to the amount of intracellular oxidant. Briefly, 4 h after K/S withdrawal (in the presence or absence of 3 mg/ml C-phycocyanin), cells were loaded with 10 µM DCF diacetate for 30 min at 37 °C. Then, cells were collected by pipetting and analysed with an Epics XL flow cytometer. The total number of cells was 5,000 per sample.

Statistics

Data are expressed as mean±SEM and the significance of differences between means was assessed by ANOVA followed by the Tukey-Kramer test. $P<0.05$ was regarded as significant.

Results

Viability and neuroprotective studies

The neuroprotective activity of C-phycocyanin in cultured CGC was evaluated after 24 h K/S deprivation. K/S withdrawal induced 60.3±3.8% neuronal death as demonstrated by neutral red staining (Fig. 1). C-phycocyanin prevented cell death after K/S withdrawal in a concentration-dependent manner (Fig. 1). The neuroprotective activity of C-phycocyanin was higher at a concentration of 3 mg/ml.

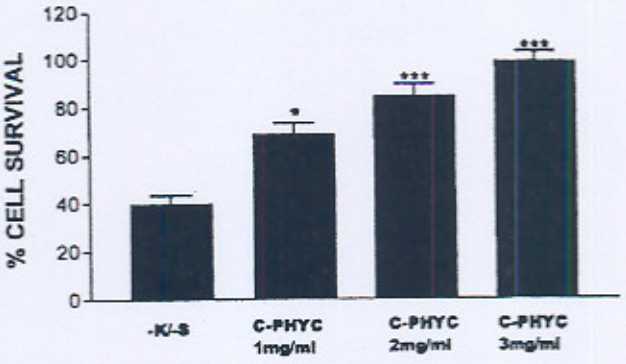

Fig. 1 Protective effect of C-phycocyanin against the toxicity accompanying cell culture in the absence of serum and with reduced [K^+] (K/S deprivation). Cerebellar granule cells (CGC) were pretreated 24 h with different concentrations of C-phycocyanin prior to K/S deprivation. Cell survival is expressed as the percentage change relative to control cells (100%). Means±SEM, n=4–6 independent experiments in quadruplicate. *$P<0.05$, ***$P<0.001$ vs. K/S-deprived medium

The neuroprotective activity of C-phycocyanin was confirmed by laser scanning cytometry using PI. Cell morphology was assessed routinely during the analyses using a CCD camera. In control preparations 7.2±1.7% (n=5) of cells were PI positive, whereas 44.7±4.0% (n=5) of K/S-deprived cells and 1.5±0.3% of K/S-deprived cells treated with C-phycocyanin (3 mg/ml, n=5) were PI positive (Fig. 2).

To address further the mechanism of K/S deprivation-induced toxicity and the neuroprotective effect of C-phycocyanin, changes in CGC morphology were examined following different experimental conditions. The loss of cell viability after 24 h K/S deprivation was accompanied by morphological changes in CGC. Disruption of neuronal network and changes in cell shape were observed (Fig. 3B). In contrast, CGC pretreated with 3 mg/ml C-phycocyanin had a morphological appearance similar to that of control cultures (Fig. 3C). Figure 3 shows representative phase-contrast images of CGC using the same objective (20×) and a digital camera (DX20; Kappa).

Assessment of apoptotic features

A distinguishing feature of apoptosis is the condensation and fragmentation of nuclear chromatin. Nuclear chromatin of control CGC permeabilized and stained with PI was dimly fluorescent, with uncondensed chromatin in large nuclei (Fig. 4A). K/S deprivation resulted in the shrunken, brightly fluorescent, apoptotic nuclei seen in Fig. 4B. The number of apoptotic cells in K/S-deprived CGC treated

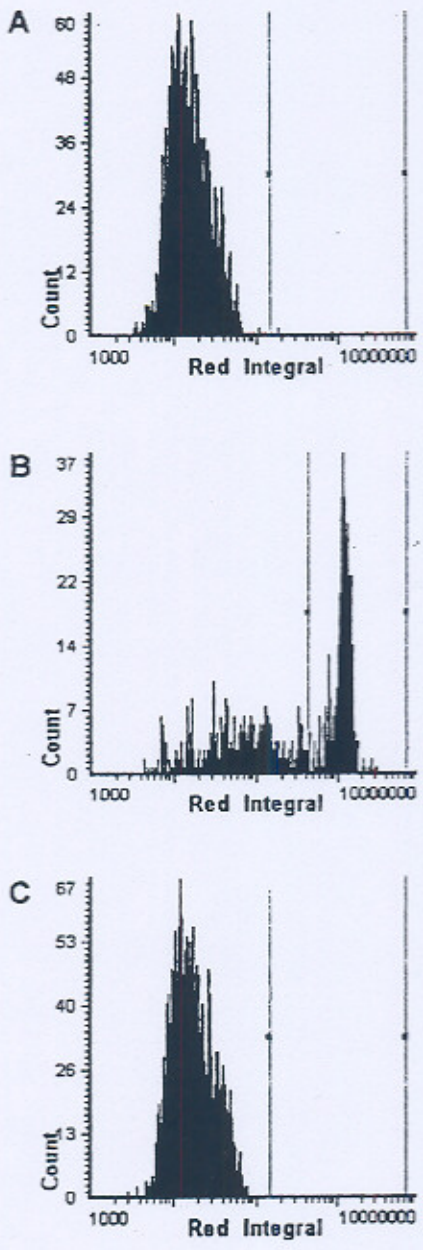

Fig. 2A–C Representative laser scanning histograms showing the neuroprotective effect of C-phycocyanin against K/S deprivation in CGC. **A** control; **B** K/S-deprived neurons; **C** K/S deprived neurons in presence of C-phycocyanin (3 mg/ml). Cells between the *vertical lines* stain positively for propidium iodide

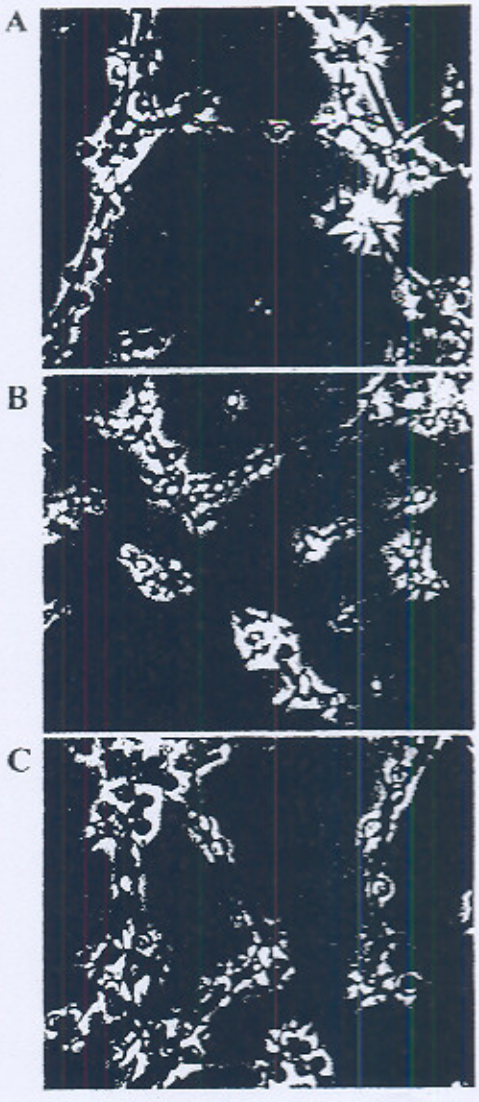

Fig. 3A–C Morphology of K/S deprivation-induced toxicity in cultured CGC. Images were taken 24 h after exposure to: **A** complete medium; **B** K/S-deprived medium; **C** K/S-deprived medium plus C-phycocyanin (3 mg/ml)

with C-phycocyanin (3 mg/ml) was reduced significantly (Fig. 4C and D).

That the mode of cell death elicited by K/S deprivation was mainly apoptotic was confirmed further by flow cytometry. The presence of a double peak on the left of Fig. 5B indicates the presence of DNA fragmentation apoptotic bodies, indicating an apoptotic process after a period of 24 h of K/S deprivation. K/S deprivation led to an 11–12% increase in the number of neurons in the S-phase, thus indicating a higher number of apoptotic cells than in non-deprived CGC (Table 1). Treatment with C-phycocyanin (3 mg/ml) 24 h before and during the K/S deprivation not only dramatically reduced the percentage of apoptotic cells, but also substantially reduced the percentage of neurons in the S-phase.

Thymidine incorporation is widely used as a measurement of cycle progression and DNA synthesis (Yan and Greene 1998). K/S deprivation increased markedly [^{3}H]methyl-thymidine incorporation (188% increase above control, Fig. 6) indicating the G_1/S progression after K/S withdrawal in CGC. Pre-treatment with 3 mg/ml C-phycocyanin prevented the increase of [^{3}H]methyl-thymidine

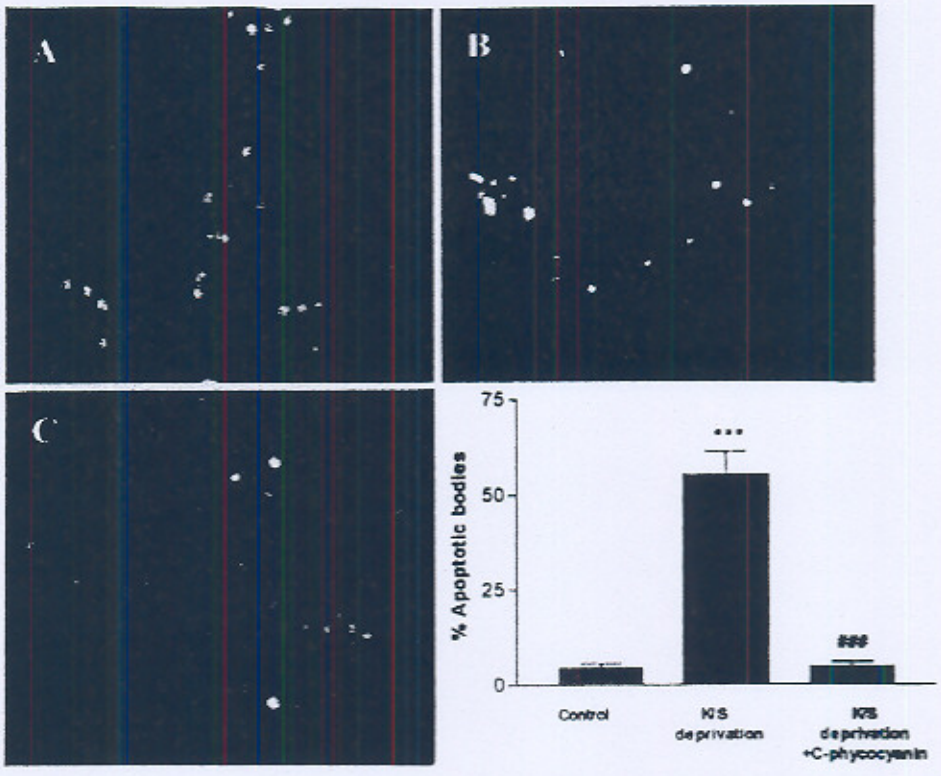

Fig. 4A–D Photomicrographs of permeabilized CGC, stained with propidium iodide to view nuclear condensation. Images were taken 24 after exposure to: **A** complete medium; **B** K/S-deprived medium; **C** K/S-deprived medium plus C-phycocyanin (3 mg/ml). **D** Bar chart showing the percentage of apoptotic bodies counted, based on the usual criteria of chromatin condensation. Nuclei from at least five separate experiments were counted. ***$P<0.001$ vs. control; ###$P<0.00$Ivs. K/S deprivation

incorporation, demonstrating the inhibition of DNA synthesis induced by K/S deprivation.

ROS generation and antioxidant properties of C-phycocyanin

K/S withdrawal increased ROS production from 1.1±0.3 (control) to 28.7±4 (arbitrary units, $n=7$, $P<0.001$). Preincubation with C-phycocyanin for 24 h prior to K/S deprivation reduced the fluorescence to 9.8±1.7 ($n=7$, $P<0.001$ vs. deprived medium). Figure 7 shows a representative histogram of DCF fluorescence under the different experimental conditions.

Discussion

Apoptosis is a form of programmed cell death which plays an important role in regulating the number of neuronal cells during the development of the central nervous system (Oppenheim 1991). The functions of apoptosis in neuronal development seem to be the removal of excess neurons and the establishment of correct synaptic connections. Recent studies have shown that apoptosis might be involved in the neuron loss that occurs in various human neurodegenerative disorders, such as Alzheimer's (Loo et al. 1993) and Huntington's disease (Portera-Cailliau et al. 1995) and amyotrophic lateral sclerosis (Rabidazeh et al. 1995).

The present study shows that the exposure of cultured CGC to K/S deprivation for 24 h is accompanied by cell death that shows, at least in part, apoptotic features. Our results are in agreement with previous studies (D'Mello et al. 1993; Galli et al. 1995; Schulz et al. 1996), which have established that K/S deprivation in CGC is an experimental model of apoptotic neuronal death in vitro.

Recent studies have suggested that neuronal apoptosis is the consequence of an inappropriate re-entry into the cell cycle (Oka et al. 1996; Park et al. 1998; Nuydens et al. 1998; Padmanabhan et al. 1999; Sakai et al. 1999; Sastry and Rao 2000). In this way it has been demonstrated that the apoptotic process induced by okadaic acid in neurons is due to an abortive mitotic attempt (Nuydens et al. 1998) and that β-amyloid protein induces an increase in the expression in cell-cycle proteins and an S-phase increase in cortical neurons (Copani et al. 1999, 2001). Moreover, kainic acid-induced apoptosis in CGC correlates with cyclin D1 expression, the latter promoting the progression from the G_1 to the S phase (Giardina et al. 1998). Stimuli such as K^+ deprivation in CGC and DNA damaging agents cause an alteration in the cell cycle and are associated with apoptotic cell death (Eldadah et al. 1997; Park et al. 1997, 1998; Padmanabhan et al. 1999). Data indicating a role for cell division cycle and apoptotic features in neurodegenerative disorders, such as Alzheimer's disease or amyotrophic lateral sclerosis (McShea et al. 1999; Raha and Robinson 2000; Zhu et al. 2000) is accumulating.

In agreement with this hypothesis, we have shown here that the apoptotic process present in K/S-deprived CGC is

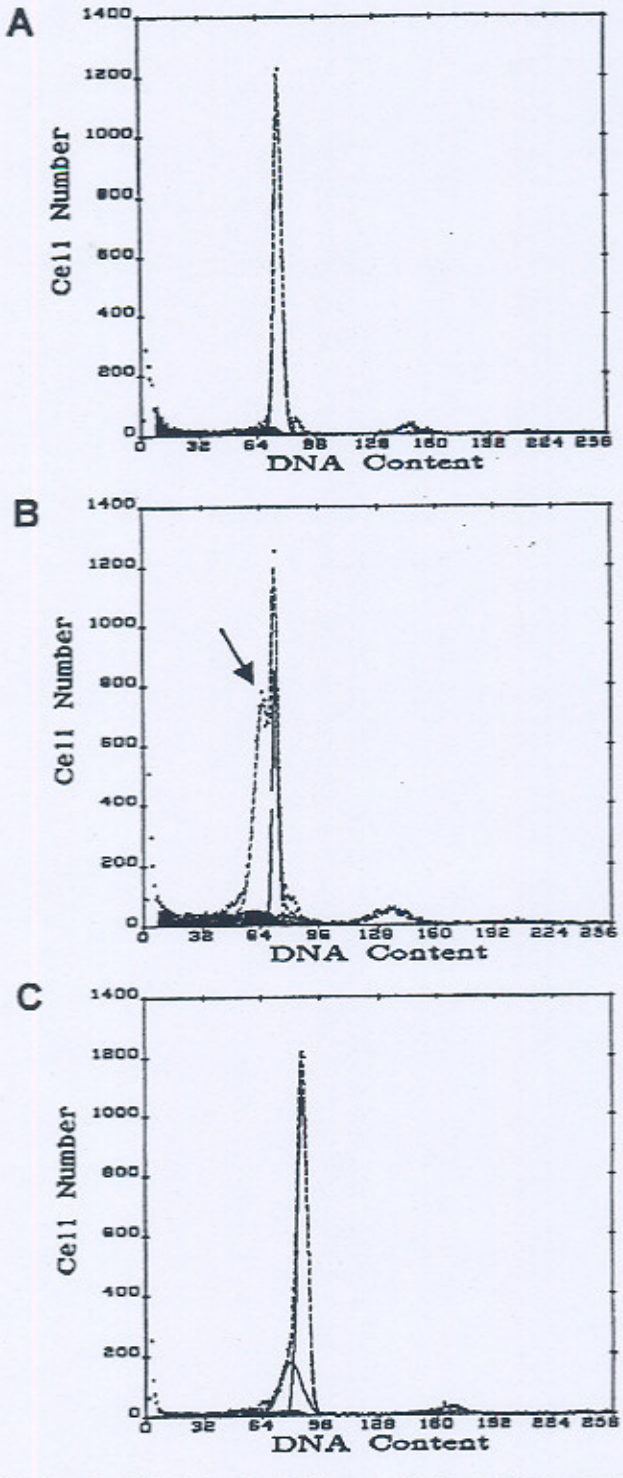

Fig. 5A–C Cell-cycle analysis (shown as fractional DNA content frequency distribution histograms) of K/S-deprived medium-induced apoptosis in CGC by flow cytometry. **A** Control; **B** K/S-deprived neurons; **C** K/S-deprived neurons in presence of C-phycocyanin (3 mg/ml). The presence of cells with fractional DNA content is indicated by the *arrow*

Table 1 Percentage fractions of apoptotic cells and cells in the various stages of the cell cycle in cultures of cerebellar granular cells obtained with Triton-100 permeabilized cells, propidium iodide staining and cytometric analysis. Means±SEM, n=5 experiments carried out in triplicate (*KS withdrawal* cell culture in the absence of serum and with reduced [K^+])

	Control	K/S withdrawal	K/S withdrawal+ C-phycocyanin (3 mg/ml)	n
Apoptosis	4.5±1	54.8±7.6	4.5±1.1***	5
G_1	91.9±2.5	78.5±4.2	91.9±2.5	5
S	1.7±1.2	13.2±3.2	1.7±1	5
G_2/M	6.4±3.6	6.4±1.9	6.4±3.6	5

***P<0.001 vs. K/S withdrawal

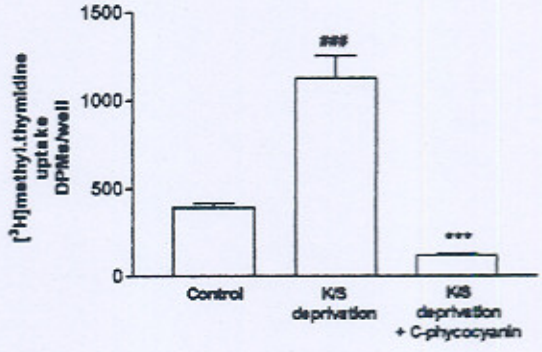

Fig. 6 Effect of C-phycocyanin on the K/S-deprived medium-induced increase in [^{3}H] methyl-thymidine incorporation in cultured rat CGC. Cell were incubated for 24 h with [^{3}H]thymidine in the presence or absence of C-phycocyanin (3 mg/ml). Means±SEM, n=5 independent experiments. ###P<0.001 vs. control; ***P<0.001 vs. deprived cultures

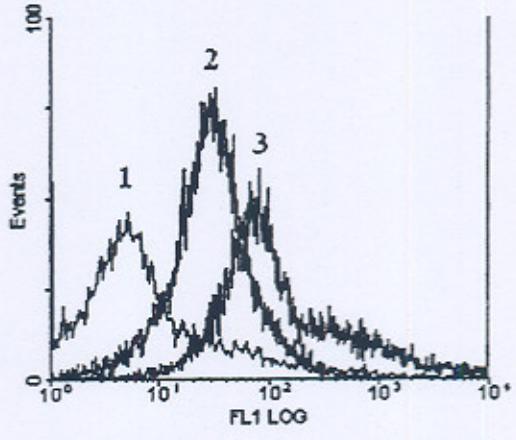

Fig. 7 Representative fluorescence histograms showing generation of reactive oxygen species (2′,7′-dichlorofluorescein fluorescence) in control neurons (*1*), the change induced by 24 h of K/S deprivation (*2*) or with C-phycocyanin 3 mg/ml (*3*)

accompanied by an increase of cells in the S-phase. Furthermore, K/S deprivation of CGC showed certain apoptotic features such as cell body shrinkage or chromatin condensation. The mechanism by which K/S deprivation initiates the cell cycle and induces cells to enter the apoptotic pathway is unknown, but one may speculate that, as in cancer cells, enhanced ROS production can induce mitotic signal transduction pathways leading to the cell cycle progression (Dreher and Junod 1996; Lin et al. 1999).

C-phycocyanin, a pigment found in blue-green algae, contains open-chain tetrapyrroles with putative scavenging properties. C-phycocyanin has anti-inflammatory activity in various in vitro and in vivo experimental models and scavenges free radicals in human leukocytes (Romay et al. 1998a, 1998b, 1999). Moreover, C-phycocyanin has a hepatoprotective action, probably by virtue of its scavenger properties (Vadarija et al. 1998). Recently, we have shown that C-phycocyanin offers protection against kainic acid-induced neurotoxicity (Rimbau et al. 1999). In the present study, using morphologic and flow cytometric analysis, we demonstrated that the apoptotic process induced by K/S withdrawal is prevented by prior C-phycocyanin treatment.

C-phycocyanin increased CGC survival after K/S deprivation and inhibited the appearance of morphological signs of apoptosis. Therefore C-phycocyanin has also neuroprotective properties in vitro.

At present, the mechanisms by which of C-phycocyanin exerts its neuroprotective effect against K/S deprivation-induced apoptosis in CGC are not clear. However, growing evidence supports the hypothesis that oxidative stress produced by different conditions can trigger apoptosis (Sastry and Rao 2000). Elevation of ROS may activate signalling pathways and expression of genes that induce apoptosis in neurons (Davis et al. 2001). Little is known about the chain of events during neuronal apoptosis, but an increase in ROS is widely accepted as an early event and that constitutes an important feature involved in apoptosis in CGC (Schulz et al. 1996). Targets of ROS that contribute to cell injury are cellular macromolecules such as lipids, proteins, carbohydrates and nucleic acids. Antioxidants and thiol reductants, such as *N*-acetylcysteine, and overexpression of manganese superoxide (MnSOD) can block or delay apoptosis. Bcl-2, an endogenous protein, prevents cells from dying of apoptosis apparently by an antioxidative mechanism (Matés 2000). Several antioxidants increase cell viability after K^+ or serum deprivation of CGC (Schulz et al. 1996), and the natural antioxidant *Ginkgo biloba* extract protects CGC from hydroxyl radical-induced apoptosis (Ni et al. 1996). ROS can thus play a pivotal role in part of signal transduction pathway during apoptosis.

We thus propose that C-phycocyanin, acting as an antioxidant, inhibits apoptotic cell death by a mechanism that involves scavenging free radicals. In fact, our data support this speculation because C-phycocyanin reduces intracellular peroxide accumulation induced by K/S deprivation in CGC at similar concentrations at which C-phycocyanin also protects human leukocytes from oxidative stress in vitro (Romay et al. 1998b). By examining the morphological changes accompanying CGC death it can be demonstrated that pretreatment with C-phycocyanin prevents the appearance of apoptotic features (small and apoptotic nuclei with chromatin condensation). Moreover, C-phycocyanin dramatically reduced [^{3}H]methyl-thymidine incorporation induced by K/S withdrawal in CGC, indicating an inhibitory role of C-phycocyanin on DNA synthesis (Kimura et al. 2000). Moreover, cytometric cell cycle studies demonstrated that this compound is able to inhibit the entry of CGC into the S-phase induced by K/S deprivation.

In conclusion, our data show that C-phycocyanin prevents cell death caused by K/S deprivation in CGC. Taking into account that these microalgae are used as dietary supplements in many countries, we have provided evidence for the potential use of this biliprotein in the prevention of neurodegenerative disorders. However in agreement with other authors (Gonzalez et al. 1998) further pre-clinical and clinical studies are required in order to confirm it.

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