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l-Deprenyl, Blocking Apoptosis and Regulating Gene Expression in Cultured Retinal Neurons*

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ABSTRACT. Apoptosis is the final pathway of many forms of retinal degeneration. E1A-NR3 is an immortalized retinal cell line that manifests specific phenotypes of retinal neurons. The present study induced apoptosis in these cells by two ischemic assaults, serum deprivation and hypoxia. The results demonstrated that both the assaults decreased viable cell numbers significantly by inducing apoptosis, as revealed by viable cell count, DNA fragmentation analysis, and *in situ* labeling of apoptotic cells by the TdT-mediated dUTP-biotin nick end-labeling (TUNEL) method. *l*-Deprenyl is known to be a monoamine oxidase inhibitor, and it was found recently to have neurotrophic activities. We set out to determine the protective effect of *l*-deprenyl on retinal cells and delineate its mechanism independent of monoamine oxidase inhibition. At concentrations as low as 0.0001 and 0.001 μM, *l*-deprenyl significantly increased the numbers of surviving cells under serum-free and hypoxic conditions, respectively. This effect appeared to be dependent upon the *l*-deprenyl concentration within the range of 0.001 to 10 μM. The neurotrophic activity was via blocking apoptosis, as *l*-deprenyl decreased the fragmented DNA and the numbers of positively stained apoptotic cells under serum-free or hypoxic conditions. Using mRNA differential display, nine mRNAs were identified and confirmed by northern blot analysis to have altered expression levels at 8 hr of exposure to hypoxia. Five of them do not match any existing sequences in GenBank, whereas the other four represent known genes including c-*jun*, heat-shock protein hsp70, phosphoglycerate kinase (PGK), and calpactin I heavy chain. All of the four mRNAs were increased significantly by hypoxia. The c-jun, PGK, and calpactin mRNAs, but not hsp70, also were increased by serum withdrawal. *l*-Deprenyl partially reversed the increase in c-jun and hsp70 mRNA levels, but not in PGK and calpactin. These results suggest that *l*-deprenyl blocks apoptosis induced by hypoxia as well as by growth factor withdrawal and regulates the expression of apoptosis-related genes. BIOCHEM PHARMACOL **58**;7:1183–1190, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. apoptosis; deprenyl; mRNA differential display; gene; neuroprotection; retina

l-Deprenyl is an irreversible inhibitor of B-type MAO¶ (MAO-B) [1, 2]. It was used originally as an adjuvant drug to L-DOPA for the treatment of Parkinson's disease and later used without L-DOPA [1–3]. The drug has been shown to slow the progression of disabling symptoms and prolong the life expectancy in patients with Parkinson's disease [1, 4, 5]. In aged rats, *l*-deprenyl was found to increase the life span and improve cognitive function [6, 7]. *l*-Deprenyl also has been reported to improve the performance of patients with Alzheimer's disease [8, 9]. Evidence has shown that *l*-deprenyl rescues striatal neurons after a lethal 1,2,3,6-tetrahydro-1-methyl-4-phenylpyridine (MPTP) assault [5]. It also displayed trophic-like activity on neurons, increased neuron survival after serum withdrawal, and induced protein synthesis [10–12]. It has been reported that deprenyl can block effectively the apoptosis induced by serum withdrawal in NGF-conditioned PC12 cells, a neuron-like cell line, but not in thymocytes, suggesting that the anti-apoptotic effect is cell type-specific [11, 13]. These neuroprotective and neurorescuing effects of *l*-deprenyl seemed to be independent of the inhibition of MAO activity, as the effective concentration for the anti-apoptotic activity of *l*-deprenyl (0.1 nM) is much lower than the concentration required for the MAO inhibitory activity (K_i = 11.25 nM) [11]. This observation suggests that the anti-apoptotic effect is via a new, as yet to be identified mechanism that is unrelated to the MAO inhibition.

Recently, Buys *et al.* [14] reported that *l*-deprenyl preserves rat optic nerve axons after an acute crush injury.

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¶ *Abbreviations*: DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; HSP, heat shock protein; MAO, monoamine oxidase; PGK, phosphoglycerate kinase; RT–PCR, reverse transcription–polymerase chain reaction; SFM, serum-free medium; and TUNEL, TdT-mediated dUTP-biotin nick end-labeling.

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Moreover, Trope *et al.* [15] have observed improved visual acuity in a small number of advanced glaucoma patients after 3 months of *l*-deprenyl treatment. These studies indicate the potential of *l*-deprenyl in the treatment of degenerative ocular diseases. The mechanism underlying the neurotrophic effect on retinal neurons, however, has not been elucidated.

E1A-NR3 cells were derived from retinal neurons of rats at postnatal day 6 [16]. These cells were immortalized by retroviral-mediated gene transfer of adenovirus 12s E1A. Unlike retinoblastoma-derived cell lines, the E1A-NR3 cells showed immortalized but untransformed features such as contact-inhibited and anchorage-dependent growth [16]. The cells manifest some phenotypes of retinal neurons after immortalization, as they express several photoreceptor-specific proteins, such as interphotoreceptor retinoid-binding protein (IRBP) and recoverin, and show a variety of characteristics that are consistent with maturing retinal neurons [16, 17]. Therefore, these cells provide a good *in vitro* model for the study of retinal development and degeneration.

In the present study, we examined the mechanisms of retinal neuron injury by trophic factor withdrawal and hypoxia and the neuroprotective effect of *l*-deprenyl using E1A-NR3 cells. We also identified some apoptosis-associated genes that are regulated by *l*-deprenyl.

MATERIALS AND METHODS

Cell Culture

E1A-NR3 cells were derived from rat retina and immortalized as described by Seigel [16]. E1A-NR3 cells were grown in DMEM containing 10% FBS, 1% MEM non-essential amino acids, 1% MEM vitamins, 1% glutamine, and 1% antibiotics [16]. Cells were cultured in humidified 5% CO_2 and 95% air at 37°. The medium was changed every 3 days until the cells reached confluence. The cells were subcultured by enzymatic dissociation using 0.0625% trypsin.

Serum-Deprivation, Hypoxia, and Cell Count

E1A-NR3 cells were seeded at a density of 1×10^5 cells/35-mm dish and cultured in DMEM containing 10% FBS at 37° in a CO_2 incubator with 5% CO_2 and 95% air. When the cells reached 70% confluence, they were subjected to serum deprivation and hypoxia experiments. For serum deprivation, the growth medium was aspirated, and the cells were washed three times with PBS. Three dishes were covered with fresh DMEM + 10% FBS as controls for serum deprivation. The remaining dishes were covered with SFM. *l*-Deprenyl was provided by Sommerset Pharmaceuticals and dissolved in PBS. It was added to the SFM to reach different concentrations, three dishes per concentration. Three dishes in the SFM received an equal volume of PBS alone as controls for the *l*-deprenyl effect. For the hypoxia experiment, the culture medium was replaced by fresh DMEM + 10% FBS, and *l*-deprenyl was added to reach different concentrations. Cells were exposed to hypoxic conditions in an incubator with a constant flow rate of a mixture of 5% CO_2 and 95% N_2 in the presence or absence of *l*-deprenyl. The three normoxic control dishes remained in 5% CO_2 and 95% air. The cells were cultured in SFM or under hypoxic conditions for 48 hr and harvested. Viable cells were counted under a light microscope with trypan blue exclusion, and differences were examined by Student's *t*-test.

DNA Fragmentation Analysis

The E1A-NR3 cells were harvested using a cell scraper. The floating cells and attached cells were combined, counted, and collected by centrifugation. Fragmented DNA was extracted according to Eastman [18]. Briefly, the cells were lysed in 5 mM Tris–Cl, pH 8.0, 0.5% Triton X-100, and 20 mM EDTA for 1 hr at 37°. After centrifugation at 27,000 *g* for 30 min, the high molecular weight DNA and cell debris in the pellet were discarded, and the fragmented DNA in the supernatant was extracted with phenol:chloroform and then precipitated with 2 vol. of cold ethanol. The DNA pellet was dissolved in 500 μL of TE buffer [10 mM Tris–Cl (pH 7.5), 10 mM EDTA]. RNase A was added to 50 μg/mL and incubated with the DNA at 37° for 1 hr. The DNA was re-extracted with phenol:chloroform and precipitated with 0.3 M sodium acetate and 70% ethanol. After centrifugation, the DNA pellet was dissolved in 30 μL of TE buffer. Low molecular weight DNA corresponding to 10^6 cells of each sample then was resolved in a 2% agarose gel and visualized by ethidium bromide staining.

Quantification of Apoptotic Cells by TUNEL

Apoptotic cells were detected using the ApopTag *in situ* Apoptosis Detection Kit-Peroxidase (Boehringer Mannheim). Cells were collected using a cell scraper and pelleted by centrifugation. The cells were washed with PBS and fixed in 100 μL of 4% neutral buffered formaldehyde for 10 min at room temperature. The cell suspension (100 μL) was dried on a glass microscope slide, and the specimens were processed according to the protocol provided by the manufacturer. The cells were counterstained with methyl green. Cells that were intact and had dark brown-stained nuclei were considered positive for apoptosis [19]. The percentage of the positively stained cells was obtained by examining 1000 cells per slide, and the mean was examined by Student's *t*-test.

mRNA Differential Display

E1A-NR3 cells were cultured to 80% confluence and then exposed to either hypoxia or normoxia for 8 hr. The cells were harvested, and total RNA was isolated by the guanidinium isothiocyanate-CsCl gradient centrifugation method as described by Sambrook *et al.* [20]. For the mRNA differential display, RNA was first cleaned with

RNase-free DNase I for 30 min at 37° to remove genomic DNA contamination. The differential display was performed using the mRNA Differential Display Kit (GenHunter Corp.) according to the manufacturer's protocol. For each reaction, 0.1 μg of total RNA was used as the template for RT followed by 30 cycles of PCR amplification. The 3′ primers are 1-base-anchored oligo-dT primers, and the 5′ primers are arbitrary primers supplied by the kit. The RT–PCR products were resolved in an 8% denaturing polyacrylamide gel. Each RT–PCR was repeated at least once using RNA from different preparations. The cDNA species showing different intensities between the normoxic and hypoxic conditions were isolated from the gel and re-amplified with PCR using the same primers. The PCR products were purified by agarose gel electrophoresis and cloned into the pCR™II vector (TA cloning kit, Invitrogen), which was introduced subsequently into *Escherichia coli* strain Top10 cells. Plasmid DNA was isolated and sequenced. The inserts were released from the vector, labeled by ^{32}P, and used as probes for northern blot analysis.

Northern Blot Analysis

Thirty micrograms of total RNA was used for northern blot analysis according to Sambrook *et al.* [20]. After hybridization with each cDNA probe, the filter containing the RNA was stripped to remove the hybridized probe and rehybridized with a housekeeping gene (β-actin) probe.

RESULTS

Effect on Cell Viability under Serum-Free or Hypoxic Conditions

As shown in Fig. 1, exposure to SFM for 48 hr significantly decreased the number of viable cells ($P < 0.01$). The addition of *l*-deprenyl at concentrations of 0.0001 to 100 μM to serum-free cultures increased cell survival significantly ($P < 0.05$ for each concentration of the drug). Cells cultured under hypoxic conditions but in serum-containing medium for 48 hr also showed significantly lower viability when compared with those under normoxic conditions ($P < 0.01$) (Fig. 1). In the presence of *l*-deprenyl, cell survival was increased significantly under the hypoxic conditions ($P < 0.05$ for each concentration of the drug except for the concentration of 0.0001 μM). The neuroprotective effect on hypoxia and serum withdrawal appeared to be dependent on the *l*-deprenyl concentration within the range of 0.001 to 10 μM (Fig. 1). At 24 hr after exposure to SFM or hypoxia, *l*-deprenyl also showed a significant protective effect on cell viability (data not shown). These results demonstrate that *l*-deprenyl protects retinal neurons from damage caused by two different assaults.

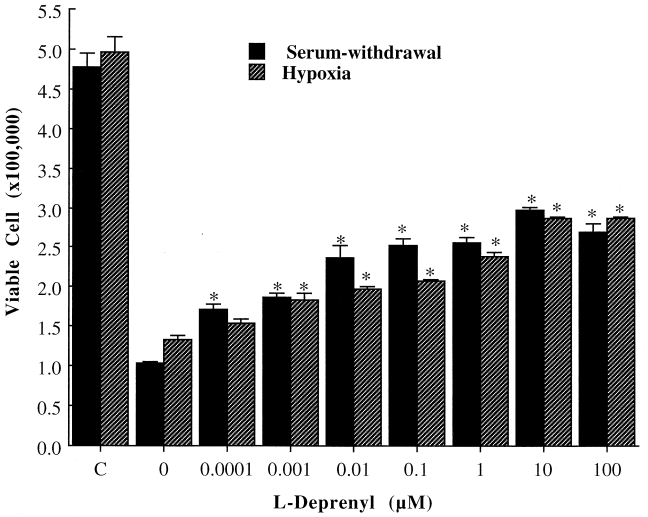

FIG. 1. Effect of *l*-deprenyl on cell viability. E1A-NR3 cells were cultured in SFM under normoxic conditions or in normal serum-containing medium under hypoxic conditions for 48 hr in the absence or presence of different concentrations of *l*-deprenyl as indicated. The viable cells were counted with trypan blue exclusion. The control group (as indicated by C) represents cells cultured in serum-containing medium and under normoxic conditions. Numbers of viable cells represent means ± SEM (N = 3). Key: (*) significantly different from the cultures without *l*-deprenyl ($P < 0.05$).

Effect on DNA Fragmentation Induced by Hypoxia or Serum Deprivation

To reveal whether the decreased cell viability by serum withdrawal and hypoxia is due to increased apoptosis, we performed DNA fragmentation analysis. In a semi-quantitative assay using low molecular weight DNA from 3×10^6 cells, control cells cultured in normal serum-containing medium and under normoxic conditions contained an undetectable level of fragmented DNA, whereas the same number of cells exposed to SFM for 24 hr showed a significant increase of fragmented DNA. In the presence of 10 or 100 μM *l*-deprenyl, the fragmented DNA was reduced markedly when the same number of cells was applied (Fig. 2, right panel). Likewise, cells exposed to hypoxia for 24 hr showed an increased DNA fragmentation that was reversed partially by *l*-deprenyl at 100 μM (Fig. 2, left panel). The results demonstrated that both serum-deprivation and hypoxia induce apoptosis in these retinal neurons. *l*-Deprenyl decreased fragmented DNA under both conditions and, thus, may have anti-apoptotic activity in retinal neurons.

Decreased Apoptotic Cells by l-Deprenyl under Serum-Free or Hypoxic Conditions

To quantify the apoptotic events, apoptotic cells were stained by labeling nicked chromosomal DNA using the TUNEL technique [21]. As shown in Fig. 3, a positively stained apoptotic cell contained a brown-stained nucleus, whereas a non-apoptotic cell had a green-stained cytoplasm but the nucleus was not stained (Fig. 3). The culture in

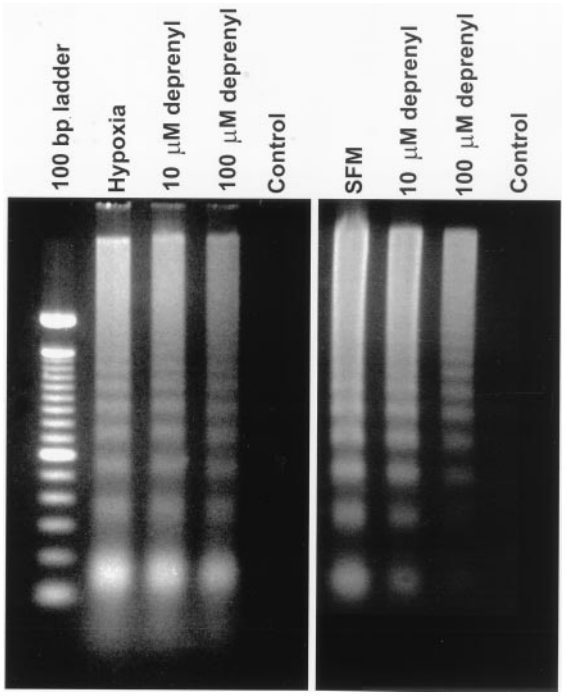

FIG. 2. Internucleosomal DNA fragmentation. Cells were cultured for 24 hr and counted. The low molecular weight DNA was isolated from the same number of cells (3×10^6) of each sample. One-third of the DNA was subjected to DNA fragmentation analysis. *l*-Deprenyl concentrations are as indicated in the figure. Left panel, fragmented DNA in cells exposed to hypoxia; Control, cells cultured under normoxic conditions; right panel, fragmented DNA in cells exposed to SFM; Control, cells in the serum-containing medium.

SFM for 24 hr contained more positively stained apoptotic cells compared with the control (Fig. 3, A and B). Fewer apoptotic cells were observed in the SFM culture with 10 μM *l*-deprenyl (Fig. 3C). The positively stained apoptotic cells were quantified as described in Materials and Methods. Cells cultured in serum-containing medium contained 6.68 ± 1.32% apoptotic cells (Fig. 3D). After exposure to SFM for 24 h, the proportion of positively stained apoptotic cells was increased to 83.33 ± 3.43% ($P < 0.01$). *l*-Deprenyl decreased apoptotic cells to 40.00 ± 1.03% ($P < 0.01$) (Fig. 3D).

Similarly, the proportion of positively stained apoptotic cells was increased from 6.68 ± 1.32% under normoxic conditions to 58.67 ± 3.33% after exposure to hypoxic conditions for 24 hr (Fig. 3E) ($P < 0.01$). The number of apoptotic cells was decreased significantly by *l*-deprenyl to 31.33 ± 2.67% under the hypoxic conditions (Fig. 3E) ($P < 0.01$). The results suggest that *l*-deprenyl blocks apoptosis induced by trophic factor withdrawal and hypoxia in retinal cells and, thus, may have a neuroprotective effect on retinal neurons.

Identification of Genes Associated with Apoptosis and Regulated by l-Deprenyl

To identify genes involved in apoptosis of retinal cells, mRNA differential display analysis was carried out. A number of mRNA species were identified that had markedly altered mRNA levels after 8 hr of exposure to hypoxia. At this time point, no apoptotic phenotypes were detected. Nine of these mRNAs were confirmed by northern blot analysis, and their sequences were determined. GenBank searches showed that five of them do not have significant sequence similarity to any existing sequences, suggesting that they may represent novel genes (data not shown); the other four match the 3′ part of known mRNAs including c-jun, heat shock protein hsp70, PGK, and calpactin I heavy chain (Fig. 4). As shown in Fig. 4A, the mRNA level of c-jun was undetectable in the control cells cultured in serum-containing medium and under normoxic conditions (Fig. 4A, lane 3). Its levels were increased markedly in the cells cultured either in SFM under normoxic conditions (Fig. 4A, lane 2) or in the serum-containing medium under hypoxic conditions (Fig. 4A, lane 6). *l*-Deprenyl (10 μM) decreased the c-jun mRNA in cells exposed to SFM (1.6-fold decrease) (Fig. 4A, lane 1) and in those exposed to hypoxia (3.5-fold decrease) (Fig. 4A, lane 5). Similarly, hsp70 mRNA was increased by hypoxia (Fig. 4B, lane 6), and the change was reversed by *l*-deprenyl (2.2-fold decrease, lane 5). Unlike c-jun, however, the hsp70 expression was not induced to a detectable level by serum deprivation (Fig. 4B, lane 2). The PGK and calpactin I mRNA levels showed changes similar to that of c-jun under the serum-free or hypoxic conditions (Fig. 4, C and D); but these changes were not reversed by *l*-deprenyl. In contrast, the β-actin mRNA did not show any detectable changes under all the conditions when the same Hybond filter was reused for hybridization (Fig. 4E), suggesting that the altered mRNA levels did not arise from the nonspecific consequences of apoptosis. Interestingly, *l*-deprenyl did not affect any of these mRNAs under normal culture conditions (Fig. 4, A–D, lane 4), indicating that it does not affect gene expression in non-apoptotic cells.

DISCUSSION

Ischemia is believed to play an important role in neuronal injury in some degenerative retinal diseases. Under ischemic conditions, neurotrophic factor deprivation and hypoxia are major factors responsible for neuron injury. To simulate ischemia, this study examined the response of retinal neurons to trophic factor withdrawal and hypoxia. The results demonstrated that cultured retinal cells die by apoptosis after an acute exposure to SFM or hypoxic atmosphere. The apoptosis induced by either of these assaults was blocked partially by *l*-deprenyl, suggesting an anti-apoptotic effect of *l*-deprenyl on retinal neurons. Multiple genes were identified to be associated with apoptosis of retinal cells and the anti-apoptotic effect of *l*-deprenyl.

A biochemical hallmark of apoptosis is the internucleosomal cleavage of chromosomal DNA into oligonucleosome-length fragments, i.e. DNA ladders [18]. This study showed significant DNA fragmentation in retinal neurons exposed to serum withdrawal and hypoxia, demonstrating

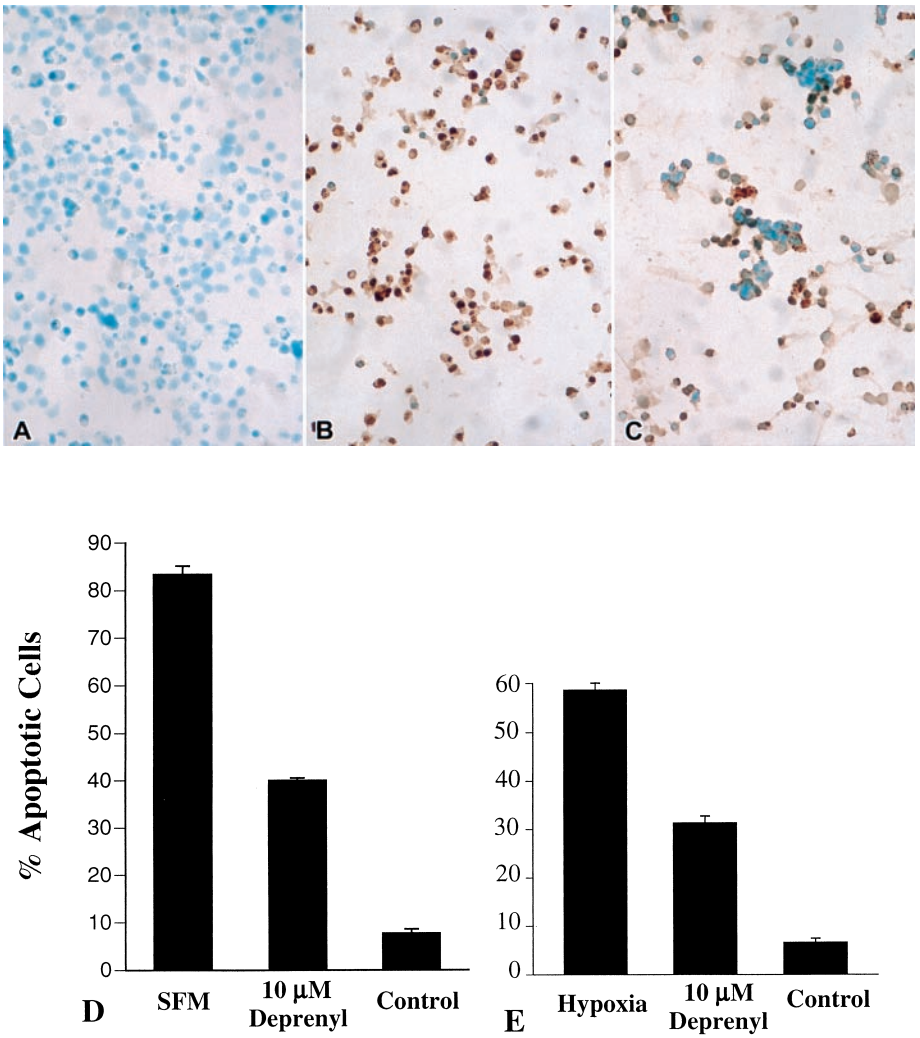

FIG. 3. Quantitation of apoptotic cells after serum deprivation or exposure to hypoxia. Cells were exposed to SFM or hypoxia for 24 hr and stained with the TUNEL method. Positively stained cells contain a dark brown nucleus, whereas non-apoptotic cells have a green-stained cytoplasm. (A) Cells cultured in serum-containing medium and under normoxic conditions. (B) Cells exposed to SFM for 24 hr under normoxic conditions. (C) Cells in SFM with 10 μM *l*-deprenyl. (D) Quantitation of positively stained cells in the serum deprivation experiments. (E) Quantitation of positively stained cells in the hypoxia experiments. The percentages of apoptotic cells represent means ± SEM (N = 3).

induced apoptosis. To obtain a better resolution of the DNA ladders, the fragmented DNA was separated from the high molecular weight DNA, and only the low molecular weight DNA was subjected to electrophoresis in the present study. Therefore, the fragmented DNA was not visible in the normal control cells, as apoptotic cells only constitute 5–6% of total cells. Although the same number of cells from each sample was used for the analysis, the cells exposed to serum withdrawal and hypoxia showed a significant amount of fragmented DNA that was reduced by *l*-deprenyl. As the DNA fragmentation analysis is not a quantitative method, the apoptotic events were quantified further by the TUNEL method, which confirmed the anti-apoptotic effect of *l*-deprenyl.

Apoptosis can be caused by different triggers and via different mechanisms depending on cell types [22–24]. It was shown recently that *l*-deprenyl reduced apoptosis induced by serum deprivation in PC12 cells, a neuron-like cell line, but not the apoptosis induced by dexamethasone in thymocytes, suggesting that its anti-apoptotic activity is dependent on the cell type and the cause of apoptosis [11, 13]. With only serum-withdrawal-induced apoptosis as a model, however, it is difficult to determine if the neuroprotective effect of *l*-deprenyl is via acting as a trophic factor supplement or through blocking the apoptotic cascade. The present study showed that either trophic factor withdrawal or hypoxia triggers apoptosis in the retinal neuronal cells, suggesting that apoptosis is the final pathway of retinal cell injury by these two different assaults. *l*-Deprenyl decreased the fragmented DNA and positively stained apoptotic cells, and increased cell survival under both conditions. These observations suggest that *l*-deprenyl not only prevents apoptosis induced by trophic factor withdrawal in retinal neurons, which is consistent with the

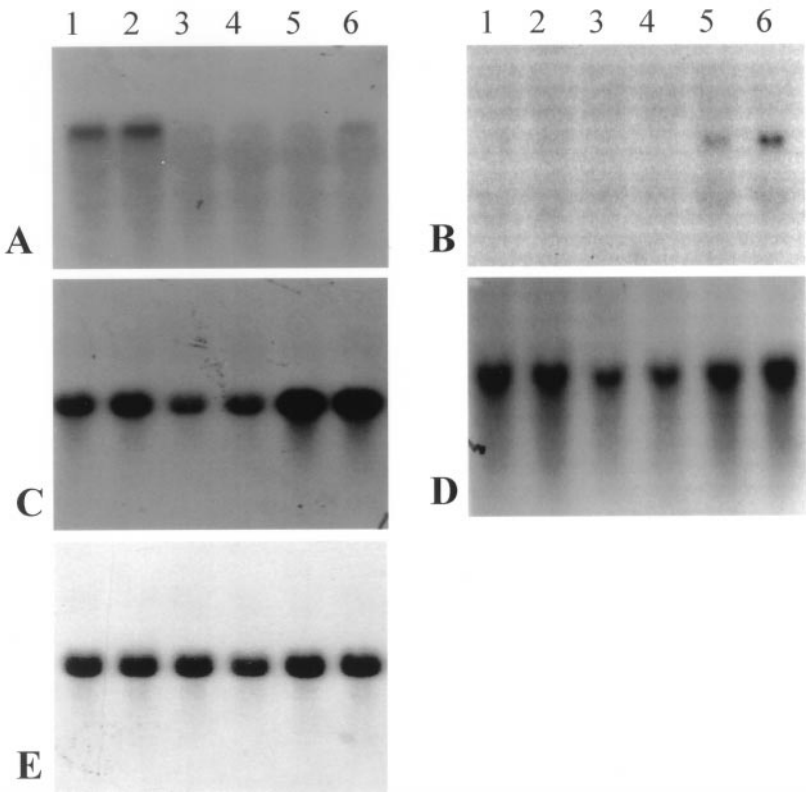

FIG. 4. Northern blot analysis of mRNAs associated with apoptosis induced by serum-withdrawal or hypoxia. Cells were exposed to either SFM or hypoxic conditions for 8 hr, and RNA was isolated. Thirty micrograms of total RNA from each condition was hybridized separately with cDNA probes of c-jun (A), hsp70 (B), PGK (C), calpactin I (D), and β-actin (E). Lane 1, SFM plus 10 μM *l*-deprenyl; 2, SFM; 3, serum-containing medium and normoxia as control; 4, control plus *l*-deprenyl; 5, hypoxia plus *l*-deprenyl; and 6, hypoxia.

finding in PC12 cells [11], but also blocks hypoxia-induced apoptosis. The observation suggests that *l*-deprenyl inhibits a common apoptosis pathway resulting from different assaults and in different cells.

As shown by viable cell count, the minimal *l*-deprenyl concentration for the protective effect on the retinal neurons is 10^{-9} M, which is significantly lower than the concentration required for MAO inhibition, supporting the notion that the anti-apoptotic effect on neuronal cells is independent of its inhibitory activity on MAO [11, 25]. Differing from necrosis, apoptosis requires gene expression and protein synthesis [12, 23]. The current study has identified a number of apoptosis-related genes that have altered expression levels at an early stage of the assaults before the appearance of any detectable apoptotic phenotypes, suggesting that the changes are unlikely to be due to the consequences of apoptosis.

Among the known genes, c-*jun* encodes an inducible transcription factor that has undetectable mRNA levels in normal neurons but is elevated significantly in apoptotic neurons [26]. It contributes directly to programmed cell death, as the up-regulation and prolonged expression of c-*jun* have been found to induce apoptosis in neurons, while suppression of c-*jun* expression prevents neuron death [23, 27]. Our results demonstrated that serum withdrawal induces higher c-*jun* expression and also more apoptotic cells compared with hypoxia (Fig. 3). These results are consistent with the previous observation that c-*jun* expression levels are correlated with the extent of apoptosis [26]. As c-jun is a transcription factor, the role of c-jun in induction of apoptosis is believed to be via regulating the transcription of target genes or through interacting with several signal cascades controlling neuronal cell death, including p53, Bax–Bcl-2, and ICE proteases [27, 28]. The present studies demonstrated that up-regulation of c-*jun* expression is an early event in apoptosis of retinal neurons and, thus, may play an important role in retinal degenerations. *l*-Deprenyl down-regulated c-*jun* expression and, thus, decreased apoptosis under either serum-free or hypoxic conditions. The results suggest that *l*-deprenyl may directly or indirectly target the c-*jun* gene and block the final signal pathway of apoptosis.

The heat shock protein hsp70 is a stress protein that functions as a chaperone by preventing the denaturing and misfolding of proteins [29]. Its role in apoptosis is uncertain even though elevated expression of hsp70 correlates with apoptosis induced by thermal stress [30]. The present study showed that hsp70 is increased significantly by hypoxia, but is not induced by serum withdrawal. The results indicate that apoptosis induced by serum withdrawal and hypoxia may have distinct molecular mechanisms or be mediated by different genes.

It has been documented that the disturbed calcium homeostasis plays an important role in ischemia-induced

cell damage [31]. Calpactin is known to bind phospholipid and actin in a calcium-dependent manner [32]. It also plays a role in the modulation of ion channels and calcium-dependent exocytosis [33, 34]. Calpactin also has been implicated in the regulation of cell proliferation, differentiation, and apoptosis [35]. Our results show that calpactin I is up-regulated markedly in both serum withdrawal and hypoxia-induced apoptosis, supporting its involvement in neuronal apoptosis.

PGK is an enzyme catalyzing the high-energy phosphoryl transfer of the acyl phosphate of 1,3-biphosphoglycerate to ADP to generate ATP. This enzyme is required for ATP generation in the glycolytic pathways [36]. Its association with neuronal apoptosis has not been documented previously. The present study revealed that PGK expression is induced markedly by both serum deprivation and hypoxia. Interestingly, the elevated PGK and calpactin mRNA levels were not reversed by *l*-deprenyl in the apoptotic retinal cells. This suggests that the gene regulation by *l*-deprenyl is a gene-specific event rather than a global effect. Identification of the apoptosis-related genes regulated by *l*-deprenyl will contribute to the understanding of the anti-apoptotic activity of *l*-deprenyl.

Apoptosis is the final pathway of retinal degeneration in a number of retinal diseases such as retinitis pigmentosa [37, 38]. It has also been reported that optic nerve degeneration is via apoptosis in some types of glaucoma [39–41]. E1A-NR3 cells are a model of retinal neurons, as they exhibit markers of retinal photoreceptor cells [16]. Although E1A-NR3 is an immortalized retinal cell line, it represents a powerful screening tool for *in vitro* drug effects, as seen in this study with *l*-deprenyl. Naturally, these results can be expanded with the use of *in vivo* models of hypoxia. However, the retinal cell line allows for tightly controlled conditions and an unlimited supply of cells for molecular studies. The induced apoptosis in these cultured retinal cells provides an *in vitro* model for studies of retinal degenerative diseases. *l*-Deprenyl blocks apoptosis and increases viability in these retinal cells exposed to different stressful conditions. Therefore, *l*-deprenyl may have neuroprotective effects on retinal neurons and may become a therapeutic agent to arrest or slow the progress of retinal degeneration.

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