Oxidative Stress Increases Production of β-Amyloid Precursor Protein and β-Amyloid (Aβ) in Mammalian Lenses, and Aβ Has Toxic Effects on Lens Epithelial Cells*

(Received for publication, December 26, 1995, and in revised form, February 7, 1996)

Peter H. Frederikse§, Donita Garlandt, J. Samuel Zigler, J.r.¶, and Joram Piatigorsky†

From the §Laboratory of Molecular and Developmental Biology and †Laboratory of Mechanisms of Ocular Diseases, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892

Many amyloid diseases are characterized by protein aggregations linked to oxidative stress. Such diseases including those of the brain, muscle, and blood vessels exhibit plaques containing β-amyloid (Aβ). Here we demonstrate that Alzheimer’s precursor protein (βAPP) and Aβ are present at low levels in normal lenses and increase in intact cultured monkey lenses treated with H2O2 or UV radiation (known cataractogenic agents), and with phorbol 12-myristate 13-acetate. AP-1 factor binding, shown by others to up-regulate βAPP expression, increased in the monkey lenses treated with H2O2, UV radiation, or phorbol 12-myristate 13-acetate and paralleled the increase in βAPP expression. Rat lenses exposed to oxidative stress showed increased βAPP in the anterior epithelium and cortex. Incubation of cultured rabbit lens N/J1003A epithelial cells with Aβ induced inclusions and vacuoles and was cytotoxic. Aβ cross-reacting protein was readily detected in the cortex of a cataractous human lens. Our data show that βAPP and Aβ increase in mammalian lenses as part of a response to H2O2 or UV radiation and suggest that they may contribute to the mechanism by which oxidative damage leads to lens opacification.

Cataract impairs vision by opacification of the ocular lens (1). A large percentage of cataracts are of the age-related (senile) type suggesting an environmental component to this degenerative disease (1–3). Both protein aggregations (4–8) and vacuole formation (9–13) are associated with several types of cataracts. Alzheimer’s protein is associated with amyloid diseases of the muscle (14, 15), brain (16, 17), and blood vessels (18), which are also characterized by protein aggregates. A pathological hallmark of Alzheimer’s disease (AD)1 is the presence of amyloid plaques, which contain β-amyloid protein (Aβ) in brain, a proteolytic cleavage product of the Alzheimer’s precursor protein (βAPP) (15, 18). In muscle fiber degeneration seen in neuromuscular diseases, including inclusion body myositis, Aβ-containing plaques are found (14, 15). Aβ plaques have also been identified in the kidneys and lungs of patients with Alzheimer’s disease (19). In the human eye, Aβ and βAPP have been detected in the retina and are associated with aging and retinal degeneration (20).

The βAPP gene is found on chromosome 21 (21) and almost all patients with trisomy 21 (Down’s syndrome) manifest AD, other Aβ plaque formation diseases, and cataracts (22–24). A 4–5-fold increase in βAPP expression over normal levels has been documented in cells from Down’s syndrome patients (25). Interestingly, a 2- and 2.5-fold increase in βAPP and Aβ, respectively, has been demonstrated in fibroblasts from non-βAPP gene duplication familial AD associated with chromosome 14 (26).

In addition to Aβ, neuritic plaques found in AD brain tissue also contain αB-crystallin, a major ocular lens protein (27). αB-Crystallin has been associated with a host of other neurodegenerative diseases (see Refs. 28 and 29 for reviews). αB-Crystallin is a small heat shock protein (30), possesses a chaperone-like anti-aggregative function (31), and mediates intermediate filament assembly in vitro (32). However, the possible role of αB-crystallin in either promoting or inhibiting amyloid filament formation is not known.

Aβ belongs to a family of amyloidogenic proteins (16), which share a strong predisposition to form insoluble β-sheet structures leading to fibrillar aggregation (33). Aβ and other amyloid proteins contain a peptide consensus repeat motif: GXX(G/S)(X)₃-X₁-X₅-(G/S)ₓ. The seeding of protein aggregate fibrillar structures by amyloid proteins like Aβ has been proposed as part of the pathogenic mechanism in amyloid disease (35).

Aβ has also been demonstrated to be cytotoxic; Aβ added exogenously to established neuronal or cerebrovascular smooth muscle cell cultures induced vacuoles and increased cell death (15, 36–40). However, when added very early in the establishment of primary neuronal cell cultures, Aβ can produce a neurotrophic effect (36). βAPP and specific cleavage products are normal constituents of many cell types and appear to be involved with facilitating membrane-associated functions (37, 38). For example, in fibroblasts, βAPP is released from cells into the medium and has an autocrine function in growth regulation (43).

Oxidative stress resulting from endogenous production of reactive oxygen species is strongly linked with amyloid disease (44, 45) and cataract (1–3). Aβ toxicity was recently shown to be mediated by H2O2 in PC12 cells (45). The potential of antioxidants in inhibiting both cataract and AD has been indicated. For example, populations with long term consumption of vitamin C (≥60 mg/day) from foods and/or supplements have reduced risks of cataract (42, 46). Experimentally, vitamin E was demonstrated to protect nerve cells from Aβ toxicity (40).

Cellular responses to oxidative stress from UV radiation and H2O2 include herpes and human immunodeficiency virus induction and increases in specific gene expression (47–50) via

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Laboratory of Molecular and Developmental Biology, NEI, National Institutes of Health, 6 Center Dr., MSC2730, Bethesda, MD 20892-2730. Tel.: 301-496-3234; Fax: 301-402-0781; E-mail: frederik@ncifcrf.gov.

The abbreviations used are: AD, Alzheimer’s disease; Aβ, β-amyloid; βAPP, Alzheimer’s precursor protein; PMA, phorbol 12-myristate 13-acetate; RT, reverse transcriptase; PCR, polymerase chain reaction.
activation of signaling pathways which involve the Ras (50) and Src (51) proteins, and include the transcription factors AP-1 (50) and NF-κB (49). A direct role for AP-1 factors in increased βAPP expression by phorbol ester (phorbol 12-myristate 13-acetate, PMA) treatment in human glial cells and HeLa cells has been described (52). PMA stimulation leads to increased AP-1 binding via a protein kinase C-mediated signal.

Results

To begin our investigation of the lenticular expression of Alzheimer’s proteins following exposure to oxidative stress, we assayed the levels of βAPP and Aβ proteins in clear intact monkey lenses. Levels of βAPP and Aβ increased in the monkey lenses following treatment with either UV254 nm radiation or H2O2, or PMA. Lenses were removed, incubated overnight to acclimate in organ culture, and treated the following morning with 1 mM H2O2, UV254 nm/8 min, or 100 ng/ml PMA and incubated for an additional 6 h unless otherwise indicated. Three cross-reacting bands (~92 kDa (βAPP), ~32 kDa, and ~4 kDa (Aβ)) increased following treatment with 1 mM H2O2 (6 or 24 h), UV, or PMA (Fig. 1). PMA has been shown elsewhere to increase βAPP expression in HeLa and glial cells through the activation of AP-1 (52). Detectable amounts of Alzheimer’s proteins in unstressed control lenses (Fig. 1, control) may reflect normal expression of this gene in the monkey lens. By contrast, an example of gene expression that is not increased by oxidative stress is the glyceraldehyde-3-phosphate dehydrogenase gene (58).

Evidence supporting expression of βAPP in untreated mammalian lenses comes from RT-dependent PCR using RNA isolated immediately after lens removal from 8-week-old FVB/N mice (Fig. 2). The presence of βAPP mRNA is indicated by the 473-base pair amplified product (Fig. 1). βAPP has been shown elsewhere to increase βAPP expression in HeLa and glial cells through the activation of AP-1 (52). Detectable amounts of Alzheimer’s proteins in unstressed control lenses (Fig. 1, control) may reflect normal expression of this gene in the monkey lens. By contrast, an example of gene expression that is not increased by oxidative stress is the glyceraldehyde-3-phosphate dehydrogenase gene (58).

Reverse Transcriptase (RT)-dependent Polymerase Chain Reaction (PCR) of Mouse βAPP—RNA was isolated from 8-week FVB/N mouse lenses. 1 µg of RNA was subjected to primer extension with the oligonucleotide primer A (2129–2109) of the mouse βAPP mRNA sequence (GenBank accession number M18373) with RT according to the manufacturer (Perkin-Elmer). The resultant cDNA was amplified by PCR according to the manufacturer (Perkin-Elmer) with primers A and B (1655–1675).

Figure 1. Western blot of proteins from rhesus monkey lenses in organ culture treated as follows: 100 ng/ml PMA for 6 h, UV254 nm for 8 min and incubated for another 6 h at 37 °C under CO2, or 1 mM H2O2 for 6 h or 24 h (see "Materials and Methods"). The blot was probed with antisera directed against Aβ.

Materials and Methods

Lentil Organ Culture—Eyes were enucleated immediately following death from Sprague-Dawley rats and rhesus monkeys (Macaca mulatta, 2–3 years of age, part of the vaccine testing program of the Center for Biologics Evaluation and Research, Food and Drug Administration). Lentil tissue was extracted and placed into 5.0 ml (monkey) or 2.0 ml (rat) of modified TC-199 medium (53, 54). Lens integrity was assessed by measuring protein leached into the medium after 30–60 min of culture, and damaged lenses were discarded (55). After equilibration under 5% CO2 at 37°C, groups of lenses were exposed to H2O2 or PMA, or to 254 nm UV radiation from an Ultraviolet Products minerallight (2 J/mm2). Lenses were homogenized with disposable pestles and sonicated in extract buffer (20 mM HEPES, 0.2 mM EDTA, 0.5 mM dithiothreitol, 450 mM NaCl, 25% glycerol, 0.5 mM leupeptin, 0.5 mM aprotinin, 0.5 mM phenylmethylsulfonyl fluoride) on ice and cleared by low speed centrifugation. Protein concentrations were estimated using a Bradford protein assay kit (Bio-Rad).

Immunohistochemistry—Human lenses were then frozen in embedding compound and immediately frozen on dry ice. Frozen sections were prepared on glass slides. Sections were fixed with 4% formalin and stained with monoclonal anti-βAPP-A4 (Boehringer Mannheim), or anti-SV40 large T-antigen monoclonal antibody (Oncogene Science). Staining was carried out with a Vectastain ABC kit (Vector) and developed using a DAB kit (Vector) according to the manufacturer.

Human eyes were obtained from the National Disease Research Interchange. Human lenses were then frozen intact in embedding compound for sectioning. Staining was carried out with a monoclonal directed against Aβ (Dako) and developed using a Vectastain ABC and DAB kits (Vector).

Tissue Culture—N1003A (56) rabbit lens epithelial cells were grown in tissue culture in Dulbecco’s modified Eagle’s medium and 10% fetal calf serum (Life Technologies, Inc.) in 30 mm plastic tissue culture dishes under 5% CO2. Cells were harvested and sonicated on ice in extract buffer as described above. Tyrophostin (30 µM) (Life Technologies, Inc.) was added as described elsewhere (50, 51) 1 h prior to treatment with H2O2.

Preparation of Aβ Peptides—Aβ1–40 and Aβ1–25–35 peptides were purchased from Bachem Bioscience Inc. The commercial peptides were prepared according to the manufacturer and diluted directly into the culture medium.

Immunoblotting—Proteins were resolved by SDS-PAGE on 18% acrylamide gels and electrophoretically transferred to Immobilon P membranes (Millipore). Following transfer, the membranes were probed with a rabbit polyclonal antibody (BMB) raised against Aβ and immune complexes were visualized with 125I-Protein A (ICN). Molecular weights were measured using SeeBlue 4–250-kDa standards (Novex).

Electrophoretic Mobility Shift Assays—Mobility shift assays were performed as described elsewhere (57) using ~40 µg of lens extract/assay tube. AP-1 consensus oligonucleotides (Santa Cruz) were used. Control experiments to test for specificity of AP-1 consensus oligonucleotides using nonspecific oligonucleotide yielded no co-migrating complex. Lens protein extracts were prepared as described above.

Reverse Transcriptase (RT)-dependent Polymerase Chain Reaction (PCR) of Mouse βAPP—RNA was isolated from 8-week FVB/N mouse lenses. 1 µg of RNA was subjected to primer extension with the oligonucleotide primer A (2129–2109) of the mouse βAPP mRNA sequence (GenBank accession number M18373) with RT according to the manufacturer (Promega). The resultant cDNA was amplified by PCR according to the manufacturer (Perkin-Elmer) with primers A and B (1655–1675).

Results

To begin our investigation of the lenticular expression of Alzheimer’s proteins following exposure to oxidative stress, we assayed the levels of βAPP and Aβ proteins in clear intact monkey lenses. Levels of βAPP and Aβ increased in the monkey lenses following treatment with either UV254 nm radiation or H2O2, or PMA. Lenses were removed, incubated overnight to acclimate in organ culture, and treated the following morning with 1 mM H2O2, UV254 nm/8 min, or 100 ng/ml PMA and incubated for an additional 6 h unless otherwise indicated. Three cross-reacting bands (~92 kDa (βAPP), ~32 kDa, and ~4 kDa (Aβ)) increased following treatment with 1 mM H2O2 (6 or 24 h), UV, or PMA (Fig. 1). PMA has been shown elsewhere to increase βAPP expression in HeLa and glial cells through the activation of AP-1 (52). Detectable amounts of Alzheimer’s proteins in unstressed control lenses (Fig. 1, control) may reflect normal expression of this gene in the monkey lens. By contrast, an example of gene expression that is not increased by oxidative stress is the glyceraldehyde-3-phosphate dehydrogenase gene (58).

Evidence supporting expression of βAPP in untreated mammalian lenses comes from RT-dependent PCR using RNA isolated immediately after lens removal from 8-week-old FVB/N mice (Fig. 2). The presence of βAPP mRNA is indicated by the RT-dependent detection of a 473-base pair amplified product using oligonucleotide primers contained within separate exons of the mouse βAPP gene. These data indicate that βAPP is present at the RNA and protein levels in monkey and mouse lenses.

Activation of the Ras and Src pathways and increases in oxidative stress is the glyceraldehyde-3-phosphate dehydrogenase gene (58).

Evidence supporting expression of βAPP in untreated mammalian lenses comes from RT-dependent PCR using RNA isolated immediately after lens removal from 8-week-old FVB/N mice (Fig. 2). The presence of βAPP mRNA is indicated by the RT-dependent detection of a 473-base pair amplified product using oligonucleotide primers contained within separate exons of the mouse βAPP gene. These data indicate that βAPP is present at the RNA and protein levels in monkey and mouse lenses.

Activation of the Ras and Src pathways and increases in oxidative stress is the glyceraldehyde-3-phosphate dehydrogenase gene (58).
beta-Amyloid and Oxidative Stress in Lens

We have presented data showing that oxidative stress can activate AP-1 factors in monkey lenses and rabbit lens epithelial cell cultures and induce beta-APP and A beta in monkey and rat lenses in organ culture. We have localized the increase in beta-APP in rat lenses to the epithelium and cortex. Moreover, A beta produces vacuoles and is toxic to cultured rabbit lens epithelial cells. We have also detected A beta cross-reacting protein in the cortex of cataractous human lenses in their opaque cortical regions. Together, these observations are consistent with the possibility that beta-amyloids may contribute to the process of cataract formation.

Many cataracts, including age-related opacities (4–8), the Nakano (59) and Emory (Ref. 60; see Ref. 61 for review) mouse hereditary mouse opacities, and x-ray induced opacities (62), are associated with the accumulation of insoluble protein aggregates. Since the ability of A beta to nucleate protein aggregation in amyloid disease is well established (33, 34, 43), it would seem from the present data that a search for the involvement of beta-amyloid proteins in nucleation events associated with cataract is warranted. The formation of heavy molecular weight lytic products on lens cell development or homeostasis require further study.

**DISCUSSION**

Weing monkey lenses or cultured N/N1003A rabbit lens epithelial cells with 100 ng/ml PMA, 1.0 mM H2O2 or UV254 nm radiation (Fig. 3). The increase in AP-1 binding paralleled the increase in beta-APP which was demonstrated in the same monkey lens extracts in Fig. 1. Increased AP-1 binding was prevented in N/N1003A cells by pretreatment with tyrophostin, an inhibitor of tyrosine phosphorylation (49) (Fig. 3). These results are consistent with the effects of H2O2 and tyrosine phosphorylation inhibitors on c-fos and c-jun expression in cultured rat lenses (58). As c-fos and c-jun expression have been shown to increase with oxidative stress in lens cells (58), our experiments do not necessarily distinguish between increased AP-1 activation and increased AP-1 factor synthesis.

We next localized the expression of beta-APP in adult rat lenses treated in organ culture for 24 h with 0, 125 , 250 , or 1.0 mM H2O2 (Fig. 4A). The immunostaining of beta-APP in H2O2-treated lenses was predominately in the anterior epithelial layer and in cortical fiber cells. Low amounts of beta-APP were also detected in untreated rat lenses and may reflect beta-APP present in normal lenses and/or base-line oxidative stress incurred during lens removal and culturing. A control SV40 T-antigen antibody showed no staining in rat lenses either with or without H2O2 treatment (data not shown). Staining of a cataractous human lens with a different monoclonal antibody directed at A beta is shown in Fig. 4B. We detected A beta in the cortical fiber cells below the epithelial cell surface layer (blue black color) with this antibody in human lenses. These results differ from H2O2-treated rat lenses, where beta-APP was detected in both the epithelial and cortical regions (Fig. 4A).

To assess the effect of A beta on cultured lens cells, we cultured N/N1003A cells in the presence of A beta-(1–40) as described elsewhere (38). Extensive inclusions and vacuoles were observed with exposure to 20 g/ml A beta-(1–40) for 24 h (Fig. 5A). In addition, <50% of the epithelial cells remained attached to the surface of the culture dish after 5 days of culture in the presence of 50 g/ml A beta-(1–40) (Fig. 5B). A beta-(25–35) peptide (15, 38) (20–50 g/ml) was cytotoxic for N/N1003A cells in culture (data not shown), producing vacuoles and decreasing cell attachment. These cytotoxic effects were produced by A beta at similar concentrations in N/N1003A cells as for neuronal cells (36). N/N1003A cells cultured in the presence of diluent alone, cytochrome c (20 g/ml), or broad range molecular weight standards (myosin, beta-galactosidase, phosphorylase b, serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, lysozyme, and aporotinin at 20 g/ml each protein; Bio-Rad) all appeared as in the control (Fig. 5A). Experiments using cells of neuronal origin have indicated that A beta-(25–35) peptide, where the amino acid order has been scrambled (40), and A beta-(1–35) peptide, where the amino acid order has been scrambled had no effect on A beta inducing its own production in cultured muscle cells (15).

**Fig. 2. Agarose gel showing products of a RT-PCR analysis of mouse beta-APP RNA.** For each reaction 1 g of freshly isolated total RNA from mouse lens was primer extended using Primer A and subsequently amplified by PCR with Primers A and B. Left lane, ethidium bromide-stained phiX174 DNA molecular weight markers (New England Biolabs); middle lane, no RT added; right lane, RT added for primer extension synthesis of cDNA.  

**Fig. 3. Electrophoretic mobility shift assays.** A, rhesus monkey lens in organ culture treated with 1.0 mM H2O2, 100 ng/ml PMA, or UV254 nm, radiation for 5 or 10 min. After treatment lenses were incubated for 6 or 24 h as indicated. B, N/N1003A cells were stimulated with 100 ng/ml PMA or 50, 125, or 250 , 1.0 mM H2O2, for 6 h. Lenses or cell extracts bound to AP1 cognate site-containing oligonucleotides (Santa Cruz) were resolved on 5% acrylamide gels. Filled arrow indicates specific complex formation, and open arrow indicates a nonspecific complex.

Tyrosine phosphorylation inhibitor tyrophostin (30) was added 1 h prior to treatment of the N/N1003A cells.
βAPP and their cleavage products are normal constituents of many cell types. However, the production of Aβ-containing proteins capable of fibril formation and/or cytotoxic effects appears to be a salient feature of amyloidogenic diseases where Aβ plays a role. The up-regulation of Aβ is most dramatic in trisomy 21 individuals (4–5-fold), where gene dosage plays a role, however, both Aβ and βAPP are also increased more than 2-fold in some familial AD involving chromosome 14, suggesting a physiological cell signaling mechanism for Aβ up-regulation as well.

The intracellular vacuoles associated with the cytotoxicity of Aβ in cultured N/N1003A lens epithelial cells in the present study are also consistent with β-amyloid proteins contributing to cataract. Intracellular vacuoles appear as a pathological hallmark in diabetes-related sugar cataracts where both osmotic (67) and oxidative (68) stresses are involved. Intracellular vacuoles appear in lens epithelial cells cultured in the presence of low concentrations of glucose and galactose (69) and appear in the central epithelium as the first detectable abnormalities during galactose cataract formation in rats (70). Similar vacuolization occurs in L-thionine sulfoximine-induced cataract in mice (71). Moreover, recent experiments on cultured rat lenses have indicated that oxidative stress caused by photochemical insult leads to vacuole formation and irreversible damage to the epithelial cells preceding and accompanying opacification (72, 73).

Since oxidative stress is considered a major cause of cataract (3, 67, 73), our finding that H2O2 and UV254 radiation increase βAPP and Aβ in cultured intact lenses supports the idea that these β-amyloid proteins play a role in cataract formation. While it has been shown that Aβ can induce H2O2 in mediating toxicity, and that anti-oxidants protect cells from Aβ toxicity in primary cultures and clonal cell lines derived from the central nervous system (44), to the best of our knowledge the present study is the first report of oxidative stress increasing Alzheimer’s proteins and is consistent with the role of AP-1 in βAPP

Fig. 4. A, immunohistochemical detection of βAPP (BMB) in rat lenses treated for 24 h. Rat lenses were placed in organ culture and H2O2 was added after 16 h culture and incubated for an additional 24 h at 37°C/5%CO2. Lenses were subsequently frozen and sectioned for antibody staining (M. A. Crawford, National Institutes of Health, Bethesda, MD). B, for comparison, staining of a cataractous human lens with a monoconal antibody directed against Aβ (DAKO) or against SV40 large T antigen (Oncogene).
gene expression. An induction of H$_2$O$_2$ by A$\beta$ could provide a feedback loop mechanism in lenses allowing A$\beta$ to increase its own expression, as has been observed in some muscle degeneration diseases (15). The ability of oxidative stress to induce A$\beta$ suggests that the manifestation of amyloid diseases could vary in different tissues depending on the amount of exposure to oxidative stress. Moreover, the proliferative-like response to oxidative stress involving such factors as AP-1 suggests that the state of growth and/or differentiation of a given cell type could also be a factor. Since our experiments suggest that both oxidative stress and phorbol ester-mediated activation of AP-1 can increase Alzheimer’s disease proteins in lens, one must consider that varied pathways and a multiplicity of signaling routes can potentially increase the ectopic expression of deleterious proteins like A$\beta$.

The mechanism of neurotoxicity attributed to the A$\beta$ peptide involves the generation of reactive oxygen species and destabilization of cellular calcium homeostasis (74). Indeed, recent experiments indicate that different amyloidogenic peptides, such as amylin, and $\beta_2$-microglobulin share this mechanism of affecting neurotoxicity (74). Thus it appears possible that in addition to a multiplicity of signaling pathways, several amyloidogenic peptides exist that potentially could give rise to toxic effects in tissues including the lens.

The mammalian lens is composed of anterior epithelial cells, which begin to elongate at the equatorial margin. These cells withdraw from the cell cycle and produce large amounts of crystallins. Terminal fiber cell differentiation is associated with the degradation of cellular organelles, has some characteristics of apoptosis (75), and involves p53 (76) and retinoblastoma (77–79) proteins. As cells in the lens cortex normally enter growth arrest, leading ultimately to nuclear breakdown and DNA fragmentation in the central region of the lens (75), we infer that an ectopic proliferative-like response to oxidative stress, which includes A$\beta$ up-regulation, may be of greater consequence than a growth arrest DNA damage response (for review see Ref. 80) to the lens. This is consistent with earlier reports showing that x-irradiation-induced cataract in amphibian lenses is greatly inhibited by preventing cell proliferation and/or differentiation through hormonal manipulation (81–83). Similarly, x-irradiation-induced cataracts do not develop in ground squirrels during hibernation when there is no lens cell proliferation (82, 83).

Oxidative stress at the levels employed in our experiments exceed that which would be experienced under normal in vivo conditions where such stress is chronic, producing effects over decades. However, low level oxidation-induced perturbations in cell signaling processes including the Ras/Src-mediated pathway for AP-1 activation (47–51) may help explain the observed link between environmental oxidative stress and cataract and possibly also AD. Studies that have shown the activation of herpes viral proliferation and skin cancers by sunlight (84) support this idea.

In summary, cataract is a disease that can involve protein aggregation and vacuole formation and thus shares similarities with AD and related A$\beta$ pathologies. Oxidative stress is an important etiological factor in these diseases and up-regulates A$\beta$ and $\beta$APP in lenses and lens cell cultures. In addition, the vacuole formation and cytotoxicity induced in cultured lens cells by A$\beta$ are similar to neuronal cell cytotoxicity demon-

FIG. 5.  

A rabbit lens epithelial N/N1003A cells in culture treated for 24 h or 42 h with 20 $\mu$g/ml A$\beta_{1-40}$ (Bachem). Control cells were cultured in solvent vehicle, cytochrome c (20 $\mu$g/ml), or broad range protein mix (Bio-Rad) at 20 $\mu$g/ml and appeared as in the control. B, N/N1003A cells cultured in the presence of 50 $\mu$g/ml A$\beta_{1-40}$ for 5 days. Cell viability was performed by counting >500 cells/unit surface area.
strated by others. Whether Aβ can elicit a trophic response in primary lens cells as it does in neuronal cells remains to be addressed. Indeed, the normal role for these proteins in neuronal as well as lens cells is not known. βAPP and Aβ proteins expressed in mammalian lenses induced by oxidative stress parallel the activation of AP-1 factor binding consistent with a role for stress-induced cell signaling in cataract formation.

Taken together, our data raise the possibility that oxidative stress, a known pathway for cataract formation, stimulates βAPP formation or aberrant βAPP protein cleavage in the ocular lens and imposes a strain on protein organization and cell integrity, contributing to lens opacification.

Acknowledgments—We thank Drs. James Kennison and Keiko Ozato for their careful reading of this manuscript and Drs. John Clark and Alex Rohrer for useful discussion. In addition, we thank Dr. Chuan Qin for assistance with protein assays of lens media and Dr. Lorenzo Segovia for advice and support during this project.

REFERENCES

1. Pitts, D. G. (1986) in Optical Radiation and Visual Health (Waxler, M., and Hitchings, V., eds) pp. 5–41, CRC Press, Inc., Boca Raton, FL

2. Hollows, F., and Moran, D. (1981) Lancet II, 1249–1254

3. Spector, A., Wang, G. M., Wang, R. R., Li, W. C., and Kuszak, J. R. (1995) Exp. Eye Res. 60, 643–649

4. Tumminia, S. J., Qin, C., Zigler, J. S., Jr., and Russell, P. (1994) Exp. Eye Res. 58, 405–410

5. Behl, C., Davis, J., Cole, G. M., and Schubert, D. (1994) Brain Res. 661, 147–156

6. Bradbury, V., J. R., and Catterall, W. A. (1994) FEBS Lett. 351, 199–203

7. Horwitz, J. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 10449–10453

8. Behl, C., Davis, J., Cole, G. M., and Schubert, D. (1992) Biochem. Biophys. Res. Commun. 186, 944–950

9. Robison, W. G., Jr., Houlder, N., and Kinoshita, J. H. (1990) Invest. Ophthalmol. Vis. Sci. 31, 793–800

10. Robison, W. G., Jr., Houlder, N., and Kinoshita, J. H. (1990) Exp. Eye Res. 50, 641–646

11. Kuettenhoff, H., and Kuettenhoff, M. (1993) Exp. Eye Res. 56, 667–677

12. Tumminia, S. J., Qin, C., Zigler, J. S., Jr., and Russell, P. (1994) Exp. Eye Res. 59, 493–498

13. Spector, A., Wang, G. M., Wang, R. R., Li, W. C., and Kleiman, N. J. (1995) Exp. Eye Res. 60, 671–674

14. Behl, C., Davis, J., Cole, G. M., and Schubert, D. (1994) Brain Res. 661, 147–156

15. Behl, C., Davis, J., Cole, G. M., and Schubert, D. (1992) Biochem. Biophys. Res. Commun. 186, 944–950

16. Muller-Hill, B., and Beyreuther, K. (1989) FEBS Lett. 253–257

17. Maury, C. P. (1995) Exp. Eye Res. 61, 515–516

18. Engler, D., Wertze, M., Kowalski, K., and Engler, M. (1995) FEBS Lett. 351, 199–203

19. Barlow, R. F., and Clow, D. (1993) Exp. Eye Res. 56, 667–677

20. Loffler, K. U., Edward, D. P., and Tso, M. O. (1995) Exp. Eye Res. 60, 641–646
Oxidative Stress Increases Production of -Amyloid Precursor Protein and -Amyloid (A) in Mammalian Lenses, and A Has Toxic Effects on Lens Epithelial Cells
Peter H. Frederikse, Donita Garland, J. Samuel Zigler, Jr. and Joram Piatigorsky

J. Biol. Chem. 1996, 271:10169-10174.
doi: 10.1074/jbc.271.17.10169

Access the most updated version of this article at http://www.jbc.org/content/271/17/10169

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 82 references, 21 of which can be accessed free at http://www.jbc.org/content/271/17/10169.full.html#ref-list-1