A Human Lens Model of Cortical Cataract: Ca\(^{2+}\)-Induced Protein Loss, Vimentin Cleavage and Opacification

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PURPOSE. Cortical cataract in humans is associated with Ca\(^{2+}\) overload and protein loss, and although animal models of cataract have implicated Ca\(^{2+}\)-activated proteases in this process, it remains to be determined whether the human lens responds in this manner to conditions of Ca\(^{2+}\) overload. The purpose of these experiments was to investigate Ca\(^{2+}\)-induced opacification and proteolysis in the organ-cultured human lens.

METHODS. Donor human lenses were cultured in Eagle's minimum essential medium (EMEM) for up to 14 days. The Ca\(^{2+}\) ionophore ionomycin was used to induce a Ca\(^{2+}\) overload. Lenses were loaded with \(^{[3]H}\)-amino acids for 48 hours. After a 24-hour control efflux period, lenses were cultured in control EMEM (Ca\(^{2+}\) 1.8 mM), EMEM + 5 \(\mu\)M ionomycin, or EMEM + 5 \(\mu\)M ionomycin + 5 mM EGTA (Ca\(^{2+}\) <1 \(\mu\)M). Efflux of proteins and transparency were monitored daily. Protein distribution and cytoskeletal proteolysis were analyzed at the end of the experiment. Cytoskeletal proteins were isolated and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Western blot analyses were probed with anti-vimentin antibody (clone V9) and detected by enhanced chemiluminescence.

RESULTS. Lenses cultured under control conditions remained transparent for 14 days in EMEM with no added supplements or serum. The lenses synthesized proteins and had a low rate of protein efflux throughout the experimental period. Ionomycin treatment resulted in cortical opacification, which was inhibited when external Ca\(^{2+}\) was chelated with EGTA. Exposure to ionomycin also led to an efflux of \(^{[3]H}\)-labeled protein, amounting to 41% of the labeled protein over the 7-day experimental period, compared with 12% in ionomycin + EGTA-treated lenses. Efflux was accounted for by loss from the lens soluble protein (crystallin) fraction. Western blot analysis of the cytoskeletal protein vimentin (56 kDa) revealed a distinct breakdown product of 48 kDa in ionomycin-treated lenses that was not present when Ca\(^{2+}\) was chelated with EGTA. In addition, high-molecular-weight proteins (~115 kDa and 235 kDa) that cross-reacted with the vimentin antibody were observed in ionomycin-treated lenses. The Ca\(^{2+}\)-induced changes were not age-dependent.

CONCLUSIONS. Human lenses can be successfully maintained in vitro, remaining transparent for extended periods. Increased intracellular Ca\(^{2+}\) induces cortical opacification in the human lens. Ca\(^{2+}\)-dependent cleavage and cross-linking of vimentin supports possible roles for calpain and transglutaminase in the opacification process. This human lens calcium-induced opacification (HLCO) model enables investigation of the molecular mechanisms of opacification, and the data help to explain the loss of protein observed in human cortical cataractous lenses in vivo. (Invest Ophthalmol Vis Sci. 2000;41:2255–2261)

Reports from early this century described ionic disruption as a characteristic occurrence in most human cataractous lenses.\(^1\) It has since been shown that although pure nuclear cataracts, accounting for approximately 30% of cataracts extracted, have a normal internal ionic content, lenses with cortical cataract (pure or mixed) have increased lenticular Na\(^{+}\) and Ca\(^{2+}\) and decreased K\(^{+}\) content.\(^2\) The ionic alterations can occur throughout the whole lens, as is the case in mature cataracts, or they can occur in highly localized regions, as is the case with retrodots and focal cortical opacities.\(^3,4\) In the case of mature cortical cataract, there is a characteristic loss of dry weight that can be attributed to a decrease in the protein content of the lens. Furthermore, the degree of disruption in the ionic balance is correlated with the loss of protein.\(^2,5\) However, an analysis of human cortical cataracts does not provide information concerning a particular role for any individual ionic species, because all ionic levels are altered to some degree.\(^2\) The ionic changes have therefore been modeled in the organ-cultured bovine lens, where loss of soluble protein and lens opacification were found to be independent of Na\(^{+}\) and K\(^{+}\) disruption and lens hydration but were critically dependent on an increase in lens Ca\(^{2+}\).\(^6\) In vivo and in vitro animal models have implicated the Ca\(^{2+}\)-activated protease calpain (EC.3.4.22.17) in the mechanism of cataractogenesis.\(^7\)

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It has recently become apparent that there are species and age differences in the response of lenses to increases in intracellular Ca$^{2+}$. The most striking difference is that in young rodent lenses (in vitro and in vivo) nuclear opacities develop in response to treatments that increase lens Ca$^{2+}$, whereas rabbit and bovine lenses, as well as older rodent lenses, undergo cortical opacification. Furthermore, human lenses are reported to contain only 3% of the calpain activity found in the rat lens, and no activity can be measured in human lens homogenates unless the endogenous inhibitor calpastatin is removed. Zigler et al. have also shown that the cultured primate lens is less sensitive to oxidative insult than the cultured rodent lens. If data from animal models are to be extrapolated to the process of cataract formation in man, information is needed from human experimental systems. Hightower and Farnum have reported that simply subjecting human lenses to elevated extracellular Ca$^{2+}$ concentration (20 mM) for 48 hours results in the appearance of discrete cortical opacities.

The present study was therefore undertaken to discover whether calcium has the same critical role to play in loss of protein from cortical cataracts that it has in animal lenses. Proteolysis and protein loss were studied by two techniques designed to increase greatly the sensitivity of the methods. Human lenses were first incubated in the presence of $[^3]$H]-amino acids to allow time for protein synthesis to occur and on exposure to conditions that would elevate internal calcium, the external medium was assayed for trichloroacetic acid (TCA)–precipitable radioactivity. Internal proteolysis was studied by Western blot methods probing for vimentin, because it is not only a critical cytoskeletal element in lens cell architecture, but it is also a recognized substrate for calcium-activated proteases such as calpain.

**METHODS**

**Lens Culture**

Donor eyes were obtained from the East Anglian Eye Bank. The research followed the tenets of the Declaration of Helsinki regarding the use of human material. After removal of the cornea for transplantation, the eyes were placed in sterile containers and covered with Eagle’s minimum essential medium (EMEM) containing 200 U/ml penicillin and 200 μg/ml streptomycin. They were stored at 4°C before dissection. Lenses were placed in culture within 48 hours after the donor’s death. As far as possible, paired lenses were used for control and experimental protocols. Because of some experimental losses it was not always possible to report all data for all lenses.

Lenses were dissected by posterior approach and incubated for 30 minutes in bicarbonate-CO$_2$–buffered EMEM (pH 7.4), containing 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin, and 50 μg/ml gentamicin. Thereafter, the lenses were maintained in EMEM with 50 μg/ml gentamicin at 35°C.

After a preculture period of 24 to 72 hours, lenses were allowed to incorporate $[^3]$H]-amino acids (Leu, Lys, Phe, Pro, and Tyr; 74 kBq/ml; Amersham, Little Chalfont, UK) into proteins during a 48-hour loading period. The end of this loading period equated to day 0 of the experimental period.

**Experimental Protocol**

During the experimental period, lens images were taken daily using a charge-coupled device (CCD) camera (UVP, Cambridge, UK) with Synoptics software (Synoptics, Cambridge, UK), and media were changed daily. After a 24-hour efflux period in control EMEM, the lenses were cultured under three experimental conditions: control EMEM (Ca$^{2+}$: 1.8 mM); EMEM + 5 μM ionomycin; EMMEM + 5 μM ionomycin + 5 mM EGTA (Ca$^{2+}$: < 1 μM). Lenses in group 3 (ionomycin + EGTA) were cultured in the EGTA medium for 30 minutes before exposure to ionomycin to chelate external Ca$^{2+}$ before introduction of the ionophore. The mean ages of lenses subjected to the various treatments did not differ significantly (Table 1). At the end of the experiment, the lenses were removed from the medium and rolled on filter paper to remove medium, adhering nonlens tissue, and vitreous humor. Wet weight was determined before lenses were frozen in liquid nitrogen. Storage was at −70°C before analysis. All media were stored at −20°C before analysis of $[^3]$H-amino acid and protein efflux.

**Analysis of Amino Acid and Protein Efflux**

The total $[^3]$H activity of efflux medium was measured by counting a 1-ml aliquot in 10 ml of scintillator (OptiPhase “SuperMix”; Wallac Scintillation Products, Milton Keynes, UK), using a liquid scintillation counter (model 1409; Wallac). Ice-cold TCA was added to 1.5-ml aliquots of medium to a final concentration of 5%, and the samples were refrigerated to precipitate the protein. The samples were centrifuged at 12 000g for 30 minutes and the supernatants counted to determine the free $[^3]$H-amino acid component of the efflux medium. The pellet was washed twice by resuspension in 5% TCA and centrifugation. Bovine serum albumin (0.2%) was added as a carrier at this stage. The pellet was then dried at 65°C, dissolved in 250 mM NaOH, and the sample counted to determine the content of $[^3]$H-labeled protein in the efflux medium. Total protein content was measured in the efflux medium using a protein assay (Coomassie Plus; Pierce & Warriner, Chester, UK).

**Analysis of Lens Proteins**

Lenses were homogenized in 1 ml of extraction buffer composed of 6 mM phosphate buffer (pH 7.2) containing 100 mM KCl, 5 mM MgCl$_2$, 10 mM 2-mercaptoethanol plus 1 mM EGTA, 1 mM EDTA, 10 μM N-ethylmaleimide, 200 μM phenylmethylsulfonyl fluoride, and 5 μM E64 to prevent proteolysis during preparation. The homogenate was centrifuged at 12,000g for 30 minutes to separate the soluble from insoluble proteins. The pellet was washed three times by resuspension in 1 ml extraction buffer and centrifugation. An aliquot of the soluble protein fraction and each of the buffer washes were treated with TCA to a final concentration of 5% to separate the soluble proteins from the free amino acid pool. The protein pellets were washed three times by resuspension in 5% TCA and centrifugation.

**Table 1. Distribution of Donor Ages and Postexperimental Wet Weights of Human Lenses**

<table>
<thead>
<tr>
<th>Experimental Protocol</th>
<th>n</th>
<th>Donor Age (y)</th>
<th>Lens Wet Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>55 ± 8 (16–76)</td>
<td>208.6 ± 13.7 (154.1–246.4)</td>
</tr>
<tr>
<td>Ionomycin</td>
<td>5</td>
<td>51 ± 13 (16–92)</td>
<td>247.9 ± 18.1 (199.6–261.7)</td>
</tr>
<tr>
<td>Ionomycin/EGTA</td>
<td>6</td>
<td>61 ± 10 (30–92)</td>
<td>234.4 ± 13.0 (210.2–283.2)</td>
</tr>
</tbody>
</table>

Data are means ± SEM (range).
gation. The washed pellets were dissolved in 250 mM NaOH for counting. The washed insoluble pellet from the lens homogenate was separated into the urea-soluble and urea-insoluble fraction by extraction in 8 M urea followed by centrifugation (12 000g for 30 minutes). The pellet was washed three times by resuspension in 4 M urea and centrifugation. The urea-insoluble pellet was dissolved in 250 mM NaOH for counting. The [3H] activity in each fraction, including all washes, was determined by scintillation counting, as described.

**Vimentin Analysis**

The urea-soluble fraction was separated by SDS-PAGE on 4% to 20% gradient gels (BioRad, Hemel Hempstead, UK). Proteins were either stained by a colloidal Coomassie blue G250 method or transferred to polyvinylidene fluoride membranes (Millipore, Watford, UK) and probed with monoclonal antibody to vimentin (clone V9; Sigma, Poole, UK). Detection was by enhanced chemiluminescence (ECL, Amersham).

Unless otherwise stated, all chemicals were obtained from Sigma. Statistical analysis was performed by paired Students t-test.

**RESULTS**

**Organ Culture of Human Lenses**

In these experiments human lenses remained transparent throughout culture in control medium (EMEM). The lenses used were from donors in the age range 16 to 92 years (Table 1). Older lenses from the beginning had increased cortical scatter and were more yellow, both characteristics of the aging lens in vivo. None of the lenses used had obvious focal cortical opacity. Figure 1 shows the appearance of lenses from 18- and 66-year-old donors cultured for 5 days.

The culture medium (EMEM) was serum free, and no supplements were found to be necessary for maintenance of transparency. However, a major criterion for successful culture was the length of the postmortem period, which had to be shorter than 48-hours for the lenses to remain viable over the 2-week culture period. All lenses showed increased light scatter in the region of the posterior suture when they were initially placed in the culture medium. However, these changes were reversible, with lenses of shorter postmortem time recovering more quickly.

A further biomarker of viability was the capacity of lenses to synthesize proteins in culture. All lenses incorporated [3H]-amino acids into protein with high consistency between paired lenses. The level of incorporation was dependent on donor age (Fig. 2), with young lenses incorporating more [3H]-amino acids into protein than older lenses. This was not due to a decline in the transport of [3H]-amino acids into the lens, because the size of the [3H]-amino acid pool did not decrease with age (data not shown). Overall, the [3H]-amino acid incorporation into protein represented 16.1% ± 1.8% of the [3H]-amino acid pool at the end of loading, whereas the values from paired lenses of the youngest donor (16 years) were 25.1% and 23.1% and from the oldest donor (92 years) were 13.1% and 9.6%, respectively.

**Lens Transparency**

Raising the intracellular Ca\(^{2+}\) by exposure to ionomycin (5 \(\mu M\)) resulted in a loss of transparency that was initiated within the first 24 hours and progressed over the experimental period. Lenses from experimental day 1 (before addition of the ionomycin), days 3 and 7 are shown (Fig. 3). Light scatter was located initially in the outermost cortical fiber cells and was most dense in the equatorial regions of the lens. The opacification progressed with time to the inner cortical fibers, but at no time was opacification of the nucleus observed. In lenses treated with ionomycin in medium in which the Ca\(^{2+}\) had been chelated with EGTA, the transparency changes were inhibited. Figure 3 shows paired lenses from a 30-year-old donor treated with ionomycin alone or ionomycin + EGTA. At day 3 the ionomycin-induced opacification was almost totally inhibited by Ca\(^{2+}\) chelation. By day 7 there was scatter in the equatorial region in ionomycin + EGTA-treated lenses, but it was restricted compared with scatter throughout the entire cortex in lenses treated with ionomycin alone.

**Lens Weights**

Lenses were weighed at the end of each experiment. There were variations due to the range of donor ages. Postexperi-
mental lens weight increased with age within each of the treatment groups. In paired lenses, ionomycin increased lens wet weight compared with control, and Ca\(^{2+}\) chelation with EGTA reduced the ionomycin-induced increase (data not shown). However, because of the age-related variations, no statistically significant differences were observed when the data were pooled (Table 1), although the means showed the same trends as the paired lenses.

**Analysis of Efflux Medium**

Exposure to ionomycin did not result in a marked perturbation of free \(^{3}H\)-amino acid efflux, and in fact a slight decrease in loss was observed over time (Fig. 4A). This was in marked contrast to the efflux of \(^{3}H\)-protein, which was very low in control lenses but greatly stimulated by exposure to ionomycin (Fig. 4B). The highest rate of loss was observed in the 24-hour control lenses but greatly stimulated by exposure to ionomycin compared with 0.07 \(\mu\)g protein, 0.33 mg protein, and external Ca\(^{2+}\) in the medium. Some redistribution of the water-insoluble fraction was observed, with a small decrease in the urea-soluble fraction (not significant) and a significant increase in the urea-insoluble fractions in ionomycin-treated lenses (\(P < 0.05\); Table 2). These changes were not significantly inhibited in the ionomycin + EGTA–treated lenses.

**Calcium-Dependent Proteolysis**

Cytoskeletal proteins are known substrates of calpain in the lens and other tissues.\(^1,2,16\) We have previously demonstrated that in whole rat and bovine lenses there is an almost total degradation of the cytoskeletal proteins spectrin, filensin and vimentin on incubation with Ca\(^{2+}\) ionophore.\(^1,7,18\) This is totally inhibited by Ca\(^{2+}\)-free medium and reduced by calpain inhibitors. Cytoskeletal proteolysis was therefore investigated in greater detail in this series of experiments to compare the response of human and rat lenses to Ca\(^{2+}\) overload.

Figure 5 shows an SDS-PAGE gel of the urea-soluble fractions isolated from a pair of 16-year-old donor lenses cultured under control conditions (C) or supplemented with 5 \(\mu\)M ionomycin (I), and a pair of 66-year-old donor lenses cultured with ionomycin (I) or ionomycin with EGTA (IE). Comparison of the protein profiles of paired lenses does not show the major proteolytic changes observed in the rat.\(^16,17\) We therefore probed for cleavage products of vimentin, which is the most rapidly degraded lens cytoskeletal protein in in vitro experiments.\(^18\) The major vimentin band was seen at 56 kDa. In addition, a number of other vimentin-reactive bands were observed in lenses from both the 16- and 66-year-old donors. Three of these were dependent on the presence of ionomycin and external Ca\(^{2+}\): a cleavage product of approximately 48 kDa and two higher molecular weight products of approxi-
mately 115 kDa and 235 kDa. These data indicate that both Ca\(^{2+}\)-dependent limited proteolysis and Ca\(^{2+}\)-dependent cross-linking occurs in the human lens.

**DISCUSSION**

The human lens is remarkably resilient when cultured in vitro. It can be maintained in a transparent state for prolonged periods in a relatively simple medium that contains neither serum nor growth factors. Furthermore, the age-related decline in lens growth\(^{19}\) is mirrored by the age-related decrease in protein-synthesis rates in cultured lenses (Fig. 2).

Calcium overload occurs in a number of diseases, including cortical cataract.\(^{20}\) The present experiments are the first to characterize the response of the human lens to intracellular Ca\(^{2+}\) overload during long-term culture with extended protocols. Paired lenses tended to be equivalent in amino acid loading, incorporation into proteins, amino acid efflux, and transparency. Variations in these parameters between pairs of lenses were as would be anticipated because of the range of donor ages. This inherent variation, however, did not limit the power of the data in statistical analysis. Differences observed between treatment groups were highly significant, clearly demonstrating that cultured human lenses can be used successfully to investigate the mechanisms of cataractogenesis.

In the human lenses, Ca\(^{2+}\) overload resulted in opacification of the lens cortex. The Ca\(^{2+}\)-induced loss of cortical transparency is consistent with data from other mammalian lenses, with the exception of the neonatal rodent lens, where a nuclear opacification is observed.\(^{7}\) The increased light scatter in the human lens was associated with an increased efflux of protein from the water-soluble protein fraction. This was shown to be a direct result of increased intracellular Ca\(^{2+}\), because chelation of the Ca\(^{2+}\) in the external medium was preventative. This human lens Ca\(^{2+}\)-induced opacification (HLCO) model reflects observed changes in human cataractogenesis. Mature human cortical cataracts have increased Ca\(^{2+}\) content and decreased dry weight.\(^{2,5}\) The latter occurs because of a loss of crystallins from the soluble fraction, by insolubilization and efflux into the aqueous humor.\(^{21,22}\) These changes were paralleled in the HLCO model, in that ionomycin induced a loss of newly synthesized soluble protein and an increase in incorporation into the water-insoluble fraction (Table 2). In addition, there was a mean increase in wet weight of approximately 20% in the lenses with Ca\(^{2+}\) overload. Because there is a concomitant loss of dry weight due to the efflux of protein from the lenses, the increase is due to increased lens hydration. Lens hydration and swelling have been observed both in animal models of cataract\(^{6,23}\) and in advanced human cortical cataract in vivo.\(^{5}\) The present model, involving an acute increase in internal calcium, produces cortical opacification within 1 week, whereas human cortical cataract may take years to develop in vivo. However, the features that the model and in vivo cataract have in common indicate that it can begin to bridge the gap between experimental animal models and human cataract.

**FIGURE 4.** (A) Efflux of [\(^{3}\)H]-labeled amino acids from control and experimental lenses. Note that the value at \(t = 0\) represents the total activity in the free amino acid pool and that after a 7-day efflux period, approximately 20% of this activity still remains within each lens. (B) Efflux of [\(^{3}\)H]-labeled proteins from the lens under the same experimental conditions as in (A). One hundred percent represents the total amount of radioactive amino acids incorporated into the protein frac-

C

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Total protein efflux (mg)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>3.0</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Each point represents mean ± SEM.
There was an interesting absence of effect of Ca\(^{2+}\) overload on the overall rate of loss of amino acids from the lens. Certainly, if a general breakdown in structure was occurring throughout the lens over the exposure period, then the \([3H]\)-amino acids, which are distributed throughout the lens, would be expected to be lost much more rapidly. They are, if anything, lost more slowly. It appears therefore that in these initial stages only the outer cortex is affected, wherein the newly synthesized (and therefore labeled) protein is located. It should be noted that less than 3% of the total protein is lost from the lens, but more than 40% of the newly synthesized protein is lost. The relatively low loss of total protein and free amino acids again indicates that internal structures are relatively intact. Indeed, we could observe no change in transparency in the nuclear regions. The small percentage change in total protein of the lens highlights the advantage of having the ability to observe changes in the newly synthesized proteins in the outer region that are first at risk. The data presented here are similar to those obtained previously from this laboratory in which an organ-cultured bovine lens model was investigated. Marcantonio et al.\(^6\) concluded that the lens behaved more as a stabilized gel system rather than simply as a collection of independent proteins encapsulated by membranes. They found that severe hydration of the lens with no increase in internal calcium, produced little protein loss, but a lesser extent of hydration accompanied by an increase in internal calcium produced a massive loss of protein, presumably by destabilizing the gel structure.

Increased intracellular Ca\(^{2+}\) results in the activation and modulation of a large number of enzymes. In relation to cataract, two families of enzymes have received particular attention: the calpains\(^7\) and the transglutaminases.\(^25\) In the human lens, calpain II activity has been investigated\(^7\) and found to be highest in the cortex of young donors and lowest in the nucleus of aged donors. Lenses also have been found to contain endogenous calpain inhibitor (calpastatin) in excess over calpain activity, and the level of calpastatin did not decrease with age.\(^9\) An excess of inhibitor over enzyme results in human lens homogenates demonstrating no calpain proteolytic activity, unless the calpastatin is removed. Using the cultured human lens, we have demonstrated the Ca\(^{2+}\)-dependent limited proteolysis of vimentin, suggesting that in vivo the lens is able to overcome the inhibition within the cell. Furthermore, the proportion of native vimentin to the Ca\(^{2+}\)-dependent vimentin breakdown product is comparable between the 16- and the 66-year-old lenses (Fig. 5B). Regulation of calpain activity within the cell is known to be modulated by several factors in addition to Ca\(^{2+}\) and calpastatin, including autolytic cleavage and phospholipids (most potently phosphatidylinositol 4,5-biphosphate \([\text{PIP}_2]\)).\(^26\) Clearly, strict regulation of protease activity is necessary to prevent unscheduled proteolysis. Although proteolytic events can be identified in the human lens, the damage appears to be limited to a greater extent than in the

### TABLE 2. Distribution of \([3H]\)-Labeled Protein in Experimental Human Lenses

<table>
<thead>
<tr>
<th>Experimental Protocol</th>
<th>n</th>
<th>([3H])-Soluble Protein (%)</th>
<th>([3H])-Urea–Soluble Protein (%)</th>
<th>([3H])-Urea–Insoluble Protein (%)</th>
<th>([3H])-Protein Efflux (%)</th>
<th>Total Protein Efflux (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>73.3 ± 4.5</td>
<td>19.8 ± 4.2</td>
<td>6.3 ± 1.4</td>
<td>0.7 ± 0.3</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Ionomycin</td>
<td>5</td>
<td>29.4 ± 5.2</td>
<td>13.3 ± 4.2</td>
<td>13.3 ± 2.6</td>
<td>40.9 ± 3.8</td>
<td>2.40 ± 0.33</td>
</tr>
<tr>
<td>Ionomycin/EGTA</td>
<td>6</td>
<td>60.0 ± 4.8</td>
<td>14.4 ± 2.0</td>
<td>13.5 ± 2.9</td>
<td>12.0 ± 2.7</td>
<td>0.22 ± 0.05</td>
</tr>
</tbody>
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**FIGURE 5.** (A) SDS-PAGE and (B) Western blot for vimentin of urea-soluble proteins from paired lenses. In both (A) and (B), lanes 1 and 2: urea-soluble proteins from control (C) and 5 μM ionomycin-treated (I) lenses from a 16-year-old donor; lanes 3 and 4: urea-soluble proteins from 5 μM ionomycin- (I) and 5 μM ionomycin + 5 mM EGTA (IE)-treated lenses from a 66-year-old donor. In (A) molecular weight markers are shown and the position of the cytoskeletal proteins spectrin, filensin and vimentin are indicated. In (B) native vimentin is marked on the left; Ca\(^{2+}\)-dependent breakdown and cross-linked products of vimentin are marked by arrows on the right.
rodent lens. This lessening of sensitivity to calpain activity in the human lens parallels the lesser sensitivity to \( \text{H}_2\text{O}_2 \) insult compared with the rodent.10

The activity of a second class of \( \text{Ca}^{2+} \)-regulated enzymes, the transglutaminases, has also been observed in the human lens,27 although most research on this enzyme has been performed using freeze-thawed or homogenized animal lenses.25,27

Vimentin has been shown to be a substrate for lens transglutaminase.28 The \( \text{Ca}^{2+} \)-dependent cross-linking of vimentin observed in the HLCO model suggests that the enzyme is activated in the human lens under conditions of intracellular \( \text{Ca}^{2+} \) overload and therefore supports a possible role for this enzyme, as well as calpain, in cataractogenesis. It will be interesting to investigate the interaction between the two \( \text{Ca}^{2+} \)-activated systems in generating light scatter in the lens.

The major objective of these experiments was to determine whether \( \text{Ca}^{2+} \)-induced opacification is observed in the human lens under physiologically relevant external \( \text{Ca}^{2+} \) concentrations. This has been clearly demonstrated. Parallels have been identified between the HLCO model and in vivo mechanisms of cataractogenesis in the human lens that may contribute to the ultimate goal of elucidation of the molecular mechanisms of human cortical cataract.

Acknowledgments

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References