Regional Differences in Store-Operated Ca^{2+} Entry in the Epithelium of the Intact Human Lens

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Purpose. An elevated level of Ca^{2+} is an important factor in cataract, yet precisely how Ca^{2+} enters the lens is unknown. Lens epithelial cells contain a range of G-protein–coupled receptors and receptor tyrosine kinases that induce increases in intracellular Ca^{2+}. Receptor-associated Ca^{2+} influx is, therefore, likely to be an important route for Ca^{2+} influx to the lens. The authors investigated stimulated and passive Ca^{2+} influx in in situ human lens epithelium.

Methods. Ca^{2+} changes in equatorial (E) and central anterior (CA) epithelial cells were monitored with the use of a Ca^{2+} indicator (Fluo4) and confocal microscopy. Gene expression was monitored by RT-PCR and immunoblotting.

Results. Adenosine triphosphate (ATP) induced Ca^{2+} responses that were smaller in CA than E. Ca^{2+} store depletion, using ATP (100 μM) or thapsigargin (1 μM), revealed greater relative store capacity and Ca^{2+} influx in E. Ca^{2+} influx was blocked by La^{3+} (0.5 μM) in both regions. Unstimulated Ca^{2+} influx was greater in E than CA. Greater expression of Orai1 and STIM1 was detected in E than in CA.

Conclusions. Greater Ca^{2+} store capacity and Ca^{2+} influx in E compared with CA reflects underlying differences in proliferation and differentiation between the regions. The relatively small resting Ca^{2+} influx in CA epithelium suggests that store-operated Ca^{2+} entry (SOCE) is the main route of Ca^{2+} influx in these cells. Greater resting influx and SOCE in E cells suggests that these are a major route for Ca^{2+} influx into the lens. Increased expression of Orai1 and STIM1 in E could account for the differences in Ca^{2+} entry. Receptor activation will modulate Ca^{2+} influx, and inappropriate activity may contribute to cortical cataract. (*Invest Ophthalmol Vis Sci.* 2009;50: 4330–4336) DOI:10.1167/iovs.08-3222

Intracellular calcium ($[Ca^{2+}]_i$) is an important modulator of cell function, and many cells, including lens cells, have a wide range of plasma membrane receptors that initiate complex Ca^{2+} signaling cascades.[1,2] In most nonexcitable cells, elevation of $[Ca^{2+}]_i$ occurs through contributions from the release of intracellular stores in the endoplasmic reticulum (ER) and influx through the plasma membrane. A variety of different types of channel, when activated, allow Ca^{2+} entry into cells. These Ca^{2+} channels include voltage-operated channels, receptor-operated channels, and second messenger–operated channels.[1] Stimulation of cells with agonists can activate G-protein (G_{q11})–coupled receptors or receptor tyrosine kinases to release Ca^{2+} from the ER store, which in turn stimulates Ca^{2+} entry from the extra-cellular medium through ubiquitous store-operated channels (SOCs).[3] Although the initial phase of receptor-induced Ca^{2+} release is important for determining the amplitude and spatial location of the signaling cascade,[1] it is only transient, and a significant fraction of the Ca^{2+} released from the ER is rapidly extruded from the cell. Not only does the more sustained Ca^{2+} entry phase allow the ER to refill, it prevents $[Ca^{2+}]_i$ from returning to basal levels during prolonged stimulation. Indeed sustained Ca^{2+} increase through store-operated Ca^{2+} entry (SOCE) plays an essential role in regulating a number of Ca^{2+}-dependent processes such as cell growth, cell-cycle progression, and apoptosis.[3] Recently, the molecular identity of SOCE was identified. The link between the filling state of the ER store and SOCE channels was established with the identification of STIM1[4] in the ER membrane and Orai1[5,6] in the plasma membrane. STIM1 is able to detect when the store is depleted of Ca^{2+} and forms into aggregations that associate with similar clusters of Orai1 in the plasma membrane, causing them to open and allow Ca^{2+} into the cell.[7] Whether by direct conformational coupling or by a Ca^{2+} influx factor, however, the precise mechanism by which STIM1 communicates with Orai1 remains controversial.[8]

To fulfill its role as a signaling molecule and to avoid the toxic effects of overload, $[Ca^{2+}]_i$ is very tightly regulated in most cells, and the concentration of free Ca^{2+} in the cytoplasm is orders of magnitude less than the extracellular environment. The Ca^{2+} concentration in aqueous humor, for example, has been measured at 1.34 mM[9] compared with a resting $[Ca^{2+}]_i$ of approximately 100 nM in lens epithelial cells.[2,10] Maintenance of Ca^{2+} homeostasis is imperative for preserving the clarity of the lens,[11] and results from previous studies have shown that lenses with cortical cataract contained an elevated level of Ca^{2+} beyond normal physiological concentrations.[12] Furthermore, the development of lens opacities has been associated with a breakdown in Ca^{2+} homeostasis and activation of the Ca^{2+}-dependent proteinase calpain.[13,14] Clearly, a detailed knowledge of Ca^{2+} homeostasis in the lens is of critical importance in understanding the processes that lead to cortical opacification. The pharmacologic profile of receptor-mediated Ca^{2+} increase in human lenses has been described in some detail,[15,16] and the importance of extracellular Ca^{2+} in shaping the responses has been demonstrated on a number of occasions.[17–19] The mechanisms underlying Ca^{2+} entry, however, have not been defined. Although circumstantial evidence indicates that voltage-operated Ca^{2+} channels exist in animal lenses,[2] they have not been demonstrated in the human lens.[17] P2X receptor-operated channels have been described in the rat lens,[20] but their functionality has yet to be determined, and their presence in the human lens is also unknown. SOCE is potentially a major route for Ca^{2+} entry. Given recent advances in our understanding of this pathway,[7] it is important to know how this mechanism is regulated in the lens.

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In this study we describe distinct differences between the quiescent cells of the central anterior epithelium and the dividing and differentiating cells of the equatorial epithelium in the characteristics of their Ca^{2+} responses to ATP. We identify significant differences in the size of the ER Ca^{2+} stores and in passive and stimulated Ca^{2+} influx between the two regions of the epithelium. We also show that both STIM1, the molecule that monitors the Ca^{2+} filling status of the ER, and Orai1, the entry channel on the plasma membrane, are present in the lens epithelium and potentially play roles in modulating Ca^{2+} entry to the lens.

Materials and Methods

Materials

2-Aminoethoxydiphenylborane was obtained from Tocris Cookson (Bristol, UK). All other compounds were from Sigma-Aldrich (Poole, Dorset, UK). Antibodies for STIM1 and ORAI1 were from ProSci Inc. (Poway, CA). Antibodies for β actin were from Cell Signaling Technology Inc. (Danvers, MA).

Native Human Lens Preparations and Measurement of Intracellular Ca^{2+} Levels by Laser Scanning Confocal Microscopy

Human lenses were obtained from donors to the East Anglian Eye Bank (Norfolk and Norwich University Hospital NHS Trust, Norwich, UK) after the cornea had been removed for transplant surgery and within 24 hours of death. Freshly dissected lenses were corralled with pins, anterior part down, in a perfusion chamber and were incubated in Eagle's modified essential medium (EMEM) containing 1 μM fluorescent dye (Fluo-4 AM; Invitrogen, Paisley, UK) for 1 hour in the dark at room temperature. The chamber was mounted on the stage of a confocal microscope (LSM 510 META; Zeiss, Oberkochen, Germany), and the anterior surface of the lens was viewed with a 10× objective. To record fluctuations in intracellular Ca^{2+}, fluorescent dye (Fluo-4 AM; Invitrogen) was excited using light at 488 nm, and emissions greater than 510 nm were collected approximately once every 2 seconds. The chamber was perfused with saline (≈1 mL/min) for the duration of the experiments, and agonists and inhibitors were added to the perfusion stream. Saline had the following composition: 130 mM NaCl, 5 mM KCl, 5 mM $NaHCO_3$, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM glucose, and 20 mM HEPES, adjusted to pH 7.25 with NaOH. The resultant image time series was analyzed (AxioVision Software; Zeiss) to extract changes in fluorescence intensity as a function of time. Images were analyzed by drawing regions of interest around all the cells in the field of view to obtain the average fluorescence intensity. Alternatively, regions of interest were drawn around individual cells. Background fluorescence was subtracted from all images. Changes in fluorescence intensity (f) as a function of time are expressed in the form $(f - f_0)/f_0$, where f_0 indicates resting fluorescence intensity.

Real-Time PCR

Freshly isolated lenses were secured with pins, anterior part uppermost, to the bottom of a culture dish in EMEM. A circular piece of central anterior epithelium (rhexis sample) was removed and frozen immediately in liquid nitrogen. The fiber mass was expelled with EMEM, adherent fibers were removed with forceps, and the remaining capsular bag containing the equatorial region epithelial cells was frozen in liquid nitrogen (capsular bag sample). Samples were stored at −80°C until required. RNA extraction and QRT-PCR were performed according to standard protocols, as previously described.[21,22] Total RNA was extracted from tissue samples with an RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. mRNA was reverse transcribed using random primers and reverse transcriptase (SuperScript II; Invitrogen) according to the manufacturer's instructions. Assays (Assays-on-Demand; Applied Biosystems, Foster City, CA) containing forward and reverse primers and the FAM-labeled probe were used for *STIM1* (hs00162394_m1) and *ORAI1* or *TMEM142A* (hs00385627_m1). PCR was performed using standard protocols (TaqMan; Applied Biosystems). All gene expression levels were normalized to the expression of *18S* (Applied Biosystems) in the same sample.

Western Blot Analysis

Protein levels were measured as previously described.[22] Cells were lysed in mammalian protein extraction reagent (MPER; Pierce, Rockford, IL) containing a protease inhibitor cocktail (Halt; Pierce) and EDTA (5 mM). Lysates were stored at −80°C until required. Samples (10 μg) were loaded onto precast 10% polyacrylamide gels (Pierce). Separated proteins were transferred to polyvinylidene difluoride membrane. After blocking for 1 hour, the membrane was incubated with the primary antibody overnight at 4°C. Secondary antibody was applied for 1 hour, and bands were detected using ECL plus Western blotting detection reagents (GE Healthcare USA, Piscataway, NJ).

Results

Agonist Responses in the Intact Lens Epithelium

Application of ATP (10 μM) to the perfusate caused an increase in $[Ca^{2+}]i$ in both regions of the epithelium (Fig. 1A). The response to ATP showed an initially rapid increase in $[Ca^{2+}]i$ that reached a peak and was followed by a slowly declining phase, giving the responses a skewed appearance (Fig. 1B). Responses recorded from individual cells also showed a rapid increase in $[Ca^{2+}]i$ that was followed by a prolonged plateau and a relatively slow decline (Fig. 1C). Most noticeable, however, was the difference in magnitude of the ATP responses between equatorial and the central anterior epithelial cells such that the Ca^{2+} increase observed in the equatorial cells was 2.5 times ($P \leq 0.01$) greater in magnitude than that recorded in central anterior cells (Fig. 1D). Single-cell responses to ATP in central anterior cells were also smaller in amplitude than responses seen in individual equatorial cells (Fig. 1C).

Capacity of the Ca^{2+} Store in Lens Epithelial Cells

One possible explanation for the difference in the magnitude of response to ATP observed between the two regions of the epithelium is that the capacity of the ER Ca^{2+} store was greater in the equatorial cells, leading to a greater release of Ca^{2+} after receptor stimulation. To measure the capacity of the Ca^{2+} store, a maximal concentration of ATP[23] was applied in the absence of extracellular Ca^{2+} (1 mM EGTA) (Fig. 2A). In this way, the contents of the Ca^{2+} stores were released while the possibility of Ca^{2+} entry from the extracellular medium was eliminated. The transitory increase in $[Ca^{2+}]_i$ that this procedure induced was a measure of the capacity of the Ca^{2+} store. When this protocol was applied to the epithelia of intact lenses, it was found that the magnitude of the Ca^{2+} increase in equatorial cells was more than twice that recorded in central anterior epithelial cells (Fig. 2C). To confirm that the measured difference in Ca^{2+} release after the application of ATP was a true reflection of store capacity, an alternative protocol was used with thapsigargin (TG) to release Ca^{2+} from the endoplasmic reticulum. TG (1 μM) inhibited the endoplasmic reticulum Ca^{2+} ATPases responsible for the reuptake of Ca^{2+} into the Ca^{2+} store and has been widely used, including by this laboratory, to deplete the Ca^{2+} stores and open SOCE pathways.[17] The dynamics of the Ca^{2+} increase that followed the application of 1 μM TG were relatively slow compared with the release caused by ATP; consequently, intracellular Ca^{2+} was elevated for a longer time (Fig. 2B). The magnitude of the Ca^{2+} increase was greater in equatorial than in central anterior

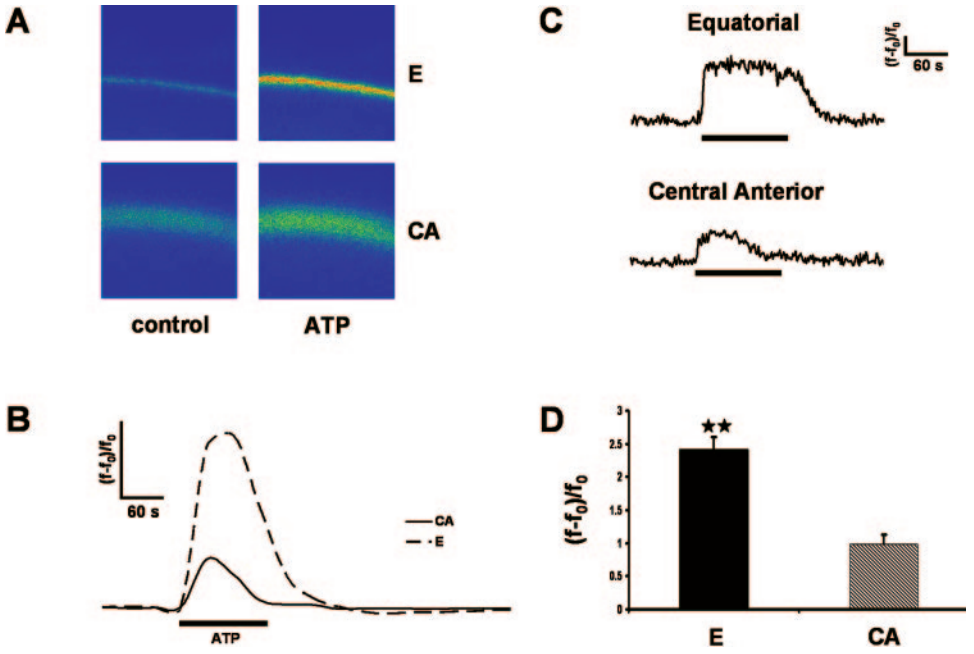

FIGURE 1. The average increase in Ca^{2+} induced by ATP was greater in the equatorial (E) than central anterior epithelium (CA). (**A**) Representative images showing the increase in Fluo4 fluorescence intensity at the equator and central anterior regions after the application of ATP (10 μM) to the lens. (**B**) Example of averaged Ca^{2+} responses from all the cells in the field of view to ATP (10 μM) recorded in the epithelium of an intact human lens. (**C**) Representative Ca^{2+} responses to ATP (10 μM) recorded in individual human lens epithelial cells. (**D**) The mean peak amplitude of Ca^{2+} increases induced by 2-minute pulses of ATP (10 μM) was greater in E than CA epithelium. Fluorescence changes are expressed as $(f - f_0)/f_0$. Values shown are mean ± SEM ($n = 5$). ★★$P \leq 0.01$ (statistically significant difference between groups determined by *t*-test).

cells when 1 μM TG was applied in Ca^{2+}-free conditions (Figs. 2B, D).

Relative Magnitude of SOCE in Equatorial and Central Anterior Lens Epithelial Cells

Another possible explanation for the differences in ATP responses between the two regions of the lens epithelium is the difference between SOCE pathways. Maximal activation of SOCE occurs when the intracellular Ca^{2+} stores are fully depleted, and this was achieved in the epithelia of intact lenses using a high dose of ATP (100 μM) or TG (1 μM) in Ca^{2+}-free medium, as previously described (Fig. 2). When Ca^{2+} was readmitted to the perfusate, the open-entry pathway allowed it to rapidly enter the cells, causing an increase in fluorescence intensity, the magnitude of which indicated the relative size of SOCE (Figs. 3A, B). With these protocols, it was found that SOCE in equatorial cells was greater than that stimulated in central anterior cells (Figs. 3C, D).

A defining characteristic of SOCE in most cells is the sensitivity to inhibition by low micromolar concentrations of trivalent cations such as lanthanum (La^{3+}).[3] Ca^{2+} influx stimulated after store depletion by ATP was rapidly blocked by La^{3+} (0.5 μM) in both regions of the lens epithelium (Fig. 4A). Ca^{2+} influx was also slowly blocked by 2-aminoethoxydiphenylborane (2APB) in E, but not CA, epithelial cells (Fig. 4B).

Resting Ca^{2+} Influx in Lens Epithelial Cells

To measure the magnitude of resting Ca^{2+} influx in the lens epithelium, Ca^{2+} in the bathing medium was increased from 1 to 10 mM for 10 minutes, and the increase in $[Ca^{2+}]_i$ was recorded. The resting influx was significantly greater ($P \leq 0.01$) in equatorial than in central anterior epithelial cells (Figs. 5A, B). Lanthanum (0.5 μM) blocked approximately 60% of the resting influx in both regions, and increasing the lanthanum concentration had no additional effect (Figs. 5C, D). There was no indication that these maneuvers stimulated Ca^{2+} entry.

Expression of *STIM1* and *ORAI1* in Native Lens Epithelial Cells

The principal components of the SOCE pathway, STIM1 and Orai1, have recently been identified.[7] We used quantitative real-time PCR (TaqMan; Applied Biosystems) to measure the expression of these genes in central anterior and equatorial epithelial cells (Figs. 6A, B). To test the purity of our samples, we checked them for expression of the fiber-specific gene

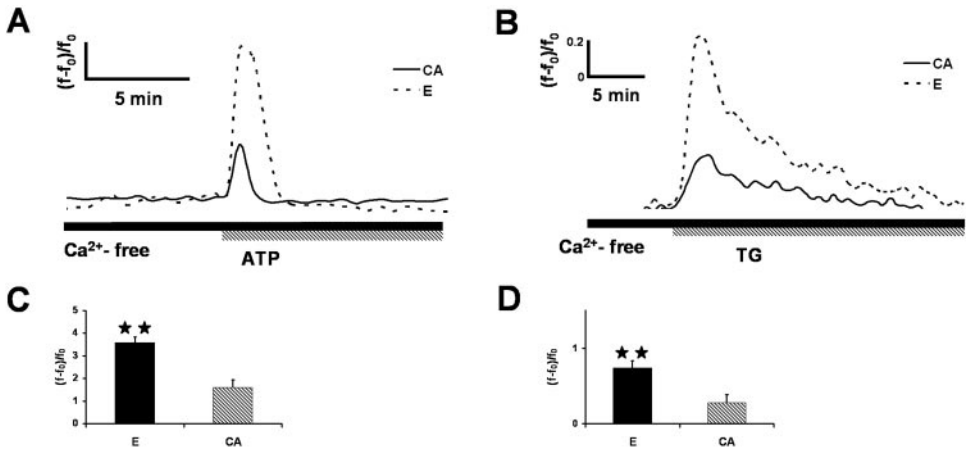

FIGURE 2. Ca^{2+} store capacity measured in intact human lens epithelium. Increases in $[Ca^{2+}]_i$ in equatorial (E) and central anterior (CA) lens epithelial cells after exposure to (**A**) ATP (100 μM) or (**B**) thapsigargin (TG; 1 μM) in Ca^{2+}-free (EGTA 1 mM) conditions. Mean relative peak fluorescence intensity after exposure to ATP (**C**) or TG (**D**) in Ca^{2+}-free conditions was greatest in E than in CA lens epithelial cells. Fluorescence changes are expressed as $(f - f_0)/f_0$. Values shown are mean ± SEM ($n = 5$). ★★$P \leq 0.01$ (statistically significant difference between groups determined by *t*-test).

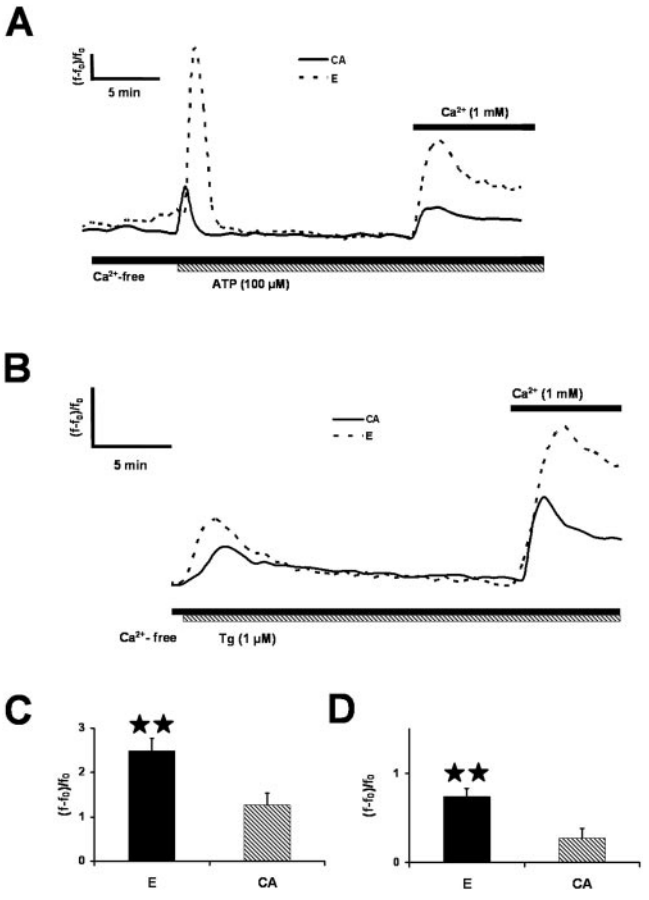

FIGURE 3. Relative magnitudes of Ca^{2+} entry pathways in different regions of the human lens epithelium were measured after store depletion by the application of either (**A**) ATP (100 μM) or (**B**) TG (1 μM). A greater SOCE was recorded in equatorial (E) than in central anterior (CA) epithelial cells when Ca^{2+} (1 mM) was readmitted to the perfusate after Ca^{2+} store depletion by ATP (**C**) or TG (**D**) in Ca^{2+}-free conditions. Fluorescence changes are expressed as $(f - f_0)/f_0$. Values shown are mean ± SEM ($n = 5$). $\star\star P \leq 0.01$ (statistically significant difference between groups determined by *t*-test).

γA-crystallin, but no expression was detected (data not shown), confirming that there was no cross-contamination with fiber cells.[24] No difference in the expression of *STIM1* between the equatorial and central anterior epithelial cells was detected (Fig. 6A). There was, however, a significantly ($P < 0.05$) greater expression of *Orai1* message in equatorial cells compared with cells in the central anterior epithelium (Fig. 6B). Protein levels were subjected to Western blot analysis in native lens samples (Fig. 6C). Orai1 migrated as a broad band with a molecular mass of approximately 47 kDa, corresponding to the glycosylated protein described by Gwack et al.[25] STIM1 migrated as a band with a molecular mass close to 90 kDa, corresponding to the glycosylated protein previously described.[26] Immunoblotting failed to detect either protein in cortical fibers.

DISCUSSION

Despite the long-established association between increased intracellular Ca^{2+} and cortical cataract,[2] the route for Ca^{2+} entry into lens cells is largely unknown. We showed in this study, however, that the major route for stimulated and passive Ca^{2+} entry into human lens epithelial cells is at the equator. The two components of Ca^{2+} signaling responsible for increasing $[Ca^{2+}]i$, release from ER Ca^{2+} stores and Ca^{2+} influx, were both greater in the equatorial than in central anterior epithelial cells. We have also shown that the SOCE components STIM1 and Orai1 were present in the epithelium and, therefore, have a potentially important role in shaping the dynamics of Ca^{2+} entry into the lens.

Regional differences in the functional activity of G-protein–coupled receptors and receptor tyrosine kinases in the human lens epithelium have been reported previously by this laboratory.[10] Analysis of ATP responses in this study, however, show that regional differences also exist in the mechanisms that underlie the receptor-induced Ca^{2+} responses. Our data indicate that regional differences in Ca^{2+} store release and Ca^{2+} influx through the plasma membrane could explain the observed differences in response size between the two regions of the epithelium. The widespread response to ATP from all cells in the epithelium, shown here and in other studies,[10] indicates that the differences in response dynamics in the two regions were probably not caused by variations in purinergic receptor distribution but instead reflect an underlying difference in the Ca^{2+} signaling pathways, a conclusion supported by the thapsigargin data. A recent study of the rat lens found low P2Y receptor expression in the epithelium compared with the cortical fibers but did not indicate regional differences.[23]

The initial peak in $[Ca^{2+}]_i$ after receptor activation was caused by Ca^{2+} release from the ER Ca^{2+} store. Here we have

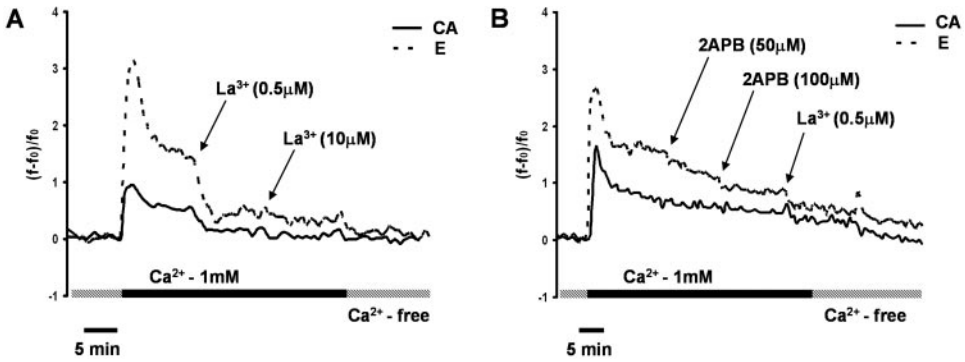

FIGURE 4. Representative recordings of SOCE in human lens epithelial cells and its inhibition by La^{3+} and 2APB. Ca^{2+} influx was stimulated by depleting Ca^{2+} stores with ATP (100 μM) in Ca^{2+}-free (EGTA 1 mM) conditions. Exposure to 1 mM Ca^{2+} caused a rapid increase in intracellular Ca^{2+} and an increase in Fluo4 fluorescence. (**A**) SOCE was blocked by La^{3+} (0.5 μM) in both epithelial regions. (**B**) SOCE was slowly blocked by 2APB (50–100 μM) in E but was not blocked in CA epithelial cells. Fluorescence changes are expressed as $(f - f_0)/f_0$. Application of inhibitors is indicated by *arrows*.

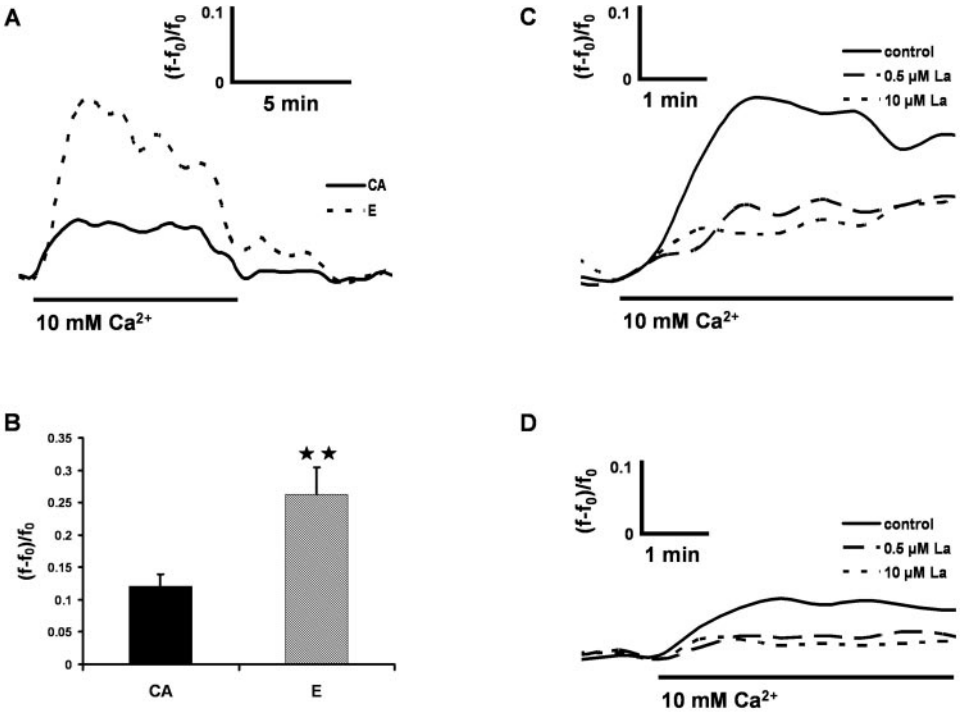

FIGURE 5. Resting Ca^{2+} influx, recorded when extracellular Ca^{2+} was increased from 1 to 10 mM. (**A**) Representative example showing greater increase in fluorescence in equatorial (E) than central anterior (CA) epithelial cells. (**B**) Summary of resting Ca^{2+} influx recordings measured in E and CA ($n = 5$; $\star\star P \leq 0.01$). Lanthanum (La) inhibited the resting Ca^{2+} influx in E (**C**) and CA (**D**) epithelial cells by a similar amount.

shown, using both maximal concentration of agonist (ATP) and the SERCA pump inhibitor TG, that the relative size of the Ca^{2+} release was smaller in the central anterior than in equatorial cells. A reduced driving force for Ca^{2+} release from the ER would be expected to have a significant effect on the magnitude of the response to agonists recorded in the central anterior region of the epithelium. It could be argued that the Ca^{2+} capacity of the stores is directly related to the protein-synthesizing activity of the cells and, hence, the relative size of the ER.[27] Therefore, the reduced Ca^{2+}-carrying capacity of the nondividing, quiescent central anterior cells likely reflects the fact that protein synthesis and, therefore, the size of the ER is reduced in these cells. The Ca^{2+} content of the ER is also important for the correct folding of newly synthesized proteins, and alterations in the Ca^{2+}-carrying capacity can indicate stress-related changes such as occur, for example, in ER stress.[28] It would, therefore, be of interest to measure Ca^{2+} signaling changes, including store capacity, in cataractous lenses in which ER stress has been a contributing factor.[29]

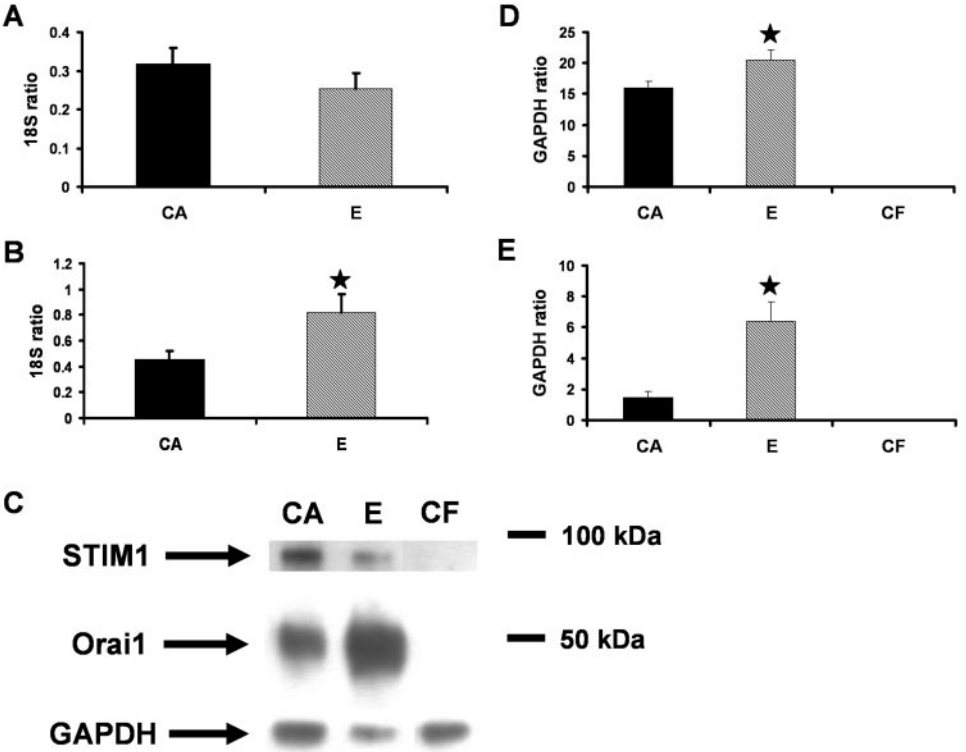

FIGURE 6. Expression of *STIM1* and *ORAI1* in native human lens epithelial samples. A disc of epithelium was taken from the central anterior (CA) of the lens. The remaining capsule, after the fiber mass had been removed, contained the equatorial epithelium (E). CF, cortical fibers. (**A**) *STIM1* mRNA levels were similar in both samples. (**B**) *ORAI1* mRNA levels were greater in E than CA samples. Mean expression levels are shown as a ratio to the expression of 18S mRNA in the samples. $n = 5$. (**C**) Western blotting detected STIM1 and Orai1 in native lens samples. Higher levels of STIM1 (**D**) and Orai1 (**E**) were detected in E than CA epithelial samples ($n = 5$). $\star P \leq 0.05$ (statistically significant difference between groups determined by *t*-test).

The other major factor that contributed to Ca^{2+} signaling responses, Ca^{2+} influx, was of greater magnitude in equatorial epithelial cells. Ca^{2+} influx was measured after Ca^{2+} stores had been depleted in Ca^{2+}-free conditions with a maximal dose of the purinergic receptor agonist ATP. This is a recognized method of activating SOCE channels,[3] and the continuous presence of ATP during this protocol ensures that the Ca^{2+} stores remain depleted throughout. As it is possible, however, that the difference in Ca^{2+} influx activated when ATP was used to deplete Ca^{2+} stores was influenced by possible differences in purinergic receptor distribution, we also measured Ca^{2+} influx when stores were depleted by thapsigargin. Ca^{2+} influx measured after the application of TG was also greater in E than in CA epithelial cells, showing a fundamental difference in the Ca^{2+}-signaling properties between the two regions. Transient receptor potential (TRP) cation channels V5 and V6 are activated by low Ca^{2+} levels,[30] and their activity could explain the increase in Ca^{2+} influx after exposure to Ca^{2+}-free conditions. However, we saw no stimulation of basal Ca^{2+} entry, in either region of the epithelium, when extracellular Ca^{2+} was removed unless intracellular stores were first depleted by the addition of ATP or TG. Unfortunately, there are no specific inhibitors of SOCE channels, though, sensitivity to La^{3+} is a fundamental characteristic of SOCE[3] and Orai1 channels are blocked by nanomolar concentrations of La^{3+} (0.5 μM).[25] Ca^{2+} influx that is blocked by La^{3+} (10 μM) is a fundamental part of the Ca^{2+} response to GPCR agonists in the lens.[21] Here we show that the Ca^{2+} influx in central anterior and equatorial cells is blocked by 0.5 μM La^{3+}, indicating the involvement of Orai1 channels. The fact that the resting influx of Ca^{2+} was also inhibited by La^{3+} (0.5 μM) suggests a constant low level of receptor or SOCE activity, even in the unstimulated epithelium. Expression of mRNA and protein for Orai1 and STIM1 in the two regions strongly indicated that these proteins are a functional component of SOCE in human lens epithelium. Significantly higher levels of STIM1 and Orai1 protein in the equatorial cells of the epithelium, provides good evidence that they account for the regional difference in the magnitude of SOCE. However, the involvement of additional SOCE components, not identified in this study, cannot be discounted.

Differences in Ca^{2+} influx in the lens epithelium may reflect differences in Ca^{2+}-ATPase, Na^+/Ca^{2+} exchange activity, or both, between the two regions because both these systems have been shown to function in the lens.[31,32] In the porcine lens, the Ca^{2+} increase induced by endothelin in epithelial cells was significantly increased in the presence of the Na^+/Ca^{2+} inhibitor bepridil or in Na^+-free conditions, indicating that Na^+/Ca^{2+} exchange can play a role in modifying Ca^{2+} signaling.[33] Although a regional difference in Na^+/Ca^{2+} exchange activity in the epithelium is a possibility, no evidence shows that this might be the case in the human lens.[2] Differential expression in the epithelium of voltage or Ca^{2+}-gated K^+ channels would alter the driving force for Ca^{2+} influx and could, therefore, be a contributory factor to explain the differences in Ca^{2+} entry. Depolarizing the membrane potential by increasing the extracellular concentration of K^+ has been shown to block Ca^{2+} influx induced by ATP in human lens cells.[17] We have previously shown that small conductance, Ca^{2+}-activated K^+ channels (SK channels) are activated during receptor stimulation in the intact human lens, and there was a greater level of message for these in the central anterior epithelium.[21] SK channel activation in the lens tends to increase the driving force for Ca^{2+} entry more in the central anterior epithelial cells and may be an adaptation to ensure efficient Ca^{2+} entry despite a reduction in the number of Ca^{2+} channels.

Until the discovery of Orai1, members of the TRPC channel family were considered the most likely candidates for SOCE.[3] TRPC channels are activated by the activity of PLC and the formation of diacylglycerol (second messenger–operated channels),[30] and the use of ATP by P2Y receptor activation to deplete Ca^{2+} stores might also have activated this class of channel. TRPC channels, however, have a lower sensitivity to block by La^{3+} than the stimulated Ca^{2+} influx described here.[34] In addition, Ca^{2+} influx through TRPC4 and TRPC5 is potentiated by micromolar La^{3+}.[30] Interestingly, recent evidence shows that TRPC channels can form heteromeric complexes with Orai1 that can be activated by STIM1 in response to Ca^{2+} store depletion.[35,36] It is, therefore, possible that differences in the inhibition of Ca^{2+} influx by 2APB are the result of differences in the makeup of the influx channels in the two regions. However, 2APB has been shown to interfere with several other processes involved in Ca^{2+} transport, including SERCA pumps, TRPV channels, and IP3 receptors,[3,37] and a degree of caution should be used when interpreting the inhibition of SOCE by 2APB. The reason why the Ca^{2+} response to acetylcholine is larger in the central anterior region[10] may also reflect differences in the channels in the two regions and the possible involvement of TRPC channels. Depleting Ca^{2+} stores with acetylcholine (100 μM), however, did not induce greater Ca^{2+} influx compared with ATP in central anterior epithelial cells (data not shown). TRPC channel expression in the lens has yet to be described, and the possible contribution to the influxes described here will be the subject of a future study.

When GPCRs were activated in equatorial epithelial cells, response characteristics were distinct from those of the central anterior epithelium because of differences in the magnitudes of both Ca^{2+} store and Ca^{2+} influx in the two regions. These differences possibly reflect greater activity in cell growth and differentiation at the lens equator. It remains to be seen, however, how these differences affect the underlying fiber cells, in which Ca^{2+} homeostasis appears to be largely under the regulation of the overlying epithelium. There is a greater degree of cell-cell communication through gap junctions between the epithelium and the underlying fibers at the equator than at the central anterior, where gap junctions are relatively sparse.[38] The greater influx of Ca^{2+} in equatorial epithelial cells might, therefore, reflect its role as a receptor-regulated conduit for Ca^{2+} to the underlying fibers, and the absence of STIM1 or Orai1 protein in the cortical fibers tends to support this view. In a recent article, however, it was shown that cortical rat lens fibers responded to GPCR agonists independently of the epithelium, suggesting that Ca^{2+} homeostasis in fiber cells is autonomous and does not involve STIM1 or Orai1.[23] Based on the extremely low resting Ca^{2+} influx in central anterior cells and in the absence of other obvious routes for Ca^{2+} influx, it can be concluded that SOCE is likely to be the major route for Ca^{2+} in this region. It can also be inferred from our data that because resting and stimulated Ca^{2+} influx is reduced in central anterior cells, the main cellular route for Ca^{2+} entry in the lens is not through these cells. Conversely, the greater resting influx and SOCE in equatorial cells is an indication that these cells are potentially a major route for Ca^{2+} entry to the lens. Under the control of extracellular signaling, SOCE may, therefore, be an important route for pathologic Ca^{2+} overload if signaling is overactive or efflux mechanisms are disturbed. The data presented here suggest that the equatorial cells of the lens are more sensitive to such disturbances and may provide a route for Ca^{2+} overload that is linked with cortical cataract.

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