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Human anterior lens capsule epithelial cells contraction

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ABSTRACT.

Purpose: **Human anterior lens epithelial cells, attached to surgically isolated capsules, were found to contract upon stimulation. The purpose of this study was to characterize these contractions, which create gaps between cells, and to assess the underlying physiological mechanisms and their possible association with cataract formation.**

Methods: **Lens capsules obtained during cataract surgery were stained with fluorescent dye Fura-2. Its fluorescence, upon excitation at 360 and 380 nm, was imaged to monitor changes in cell morphology and cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]_i$) in response to pharmacological stimulation by acetylcholine (ACh) and to mechanical stimulation by flow of saline or direct contact.**

Results: **Epithelial cells contracted in approximately a third of preparations when stimulated by either ACh application, fluid movement or direct mechanical contact. Contractions started either before or at best simultaneously with the rise in $[Ca^{2+}]_i$. Contractions also occurred when there was hardly any change in $[Ca^{2+}]_i$ upon application of physiological saline alone. The probability of contractions occurring did not differ significantly among cortical, nuclear and combined cortical + nuclear cataract.**

Conclusions: **This study provides the evidence that contractions of the anterior lens epithelial cells take place in significant portion of human lens anterior capsule postoperative preparations after non-specific stimulation. Contractions are at least partially independent of changes in $[Ca^{2+}]_i$. They can be mechanically induced, are localized and reversible and have a fast response and did not differ among different types of cataract. Physiological and clinical significance of this phenomenon remains to be elucidated.**

Key words: acetylcholine – cataract – cell contraction – Fura-2 – intracellular calcium – lens epithelial cells – mechanical stimulation

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Introduction

An important function of the ocular lens epithelium is to maintain the lens internal milieu by providing the driving force for the ionic gradients and the fluid circulation within the lens. In order to be able to perform this, its integrity is important. The epithelium is the lens layer where most constitutive elements of the mechanisms responsible for the transport of water, ions and nutrients through the lens are situated (Fischbarg et al. 1999; Bhat 2001; Delamere & Tamiya 2009). As the contractions of epithelial cells and the appearance of gaps in the monolayer would very likely influence these transport functions, they may contribute to pathological conditions such as cataract formation.

While contractions of lenses and lens epithelial cells were described in isolated chick embryo lenses (Oppitz et al. 2003), contractions of human adult lens epithelial cells were, to our knowledge, never systematically studied and described in details. The ability to contract is a property of many non-muscle cells, e.g. endothelial cells (Kolodney & Wysolmerski 1992; Mehta & Malik 2006) and many other epithelial cells (Lee & Auersperg 1980; Joshi et al. 2010). Contractions are often described to be induced by acetylcholine (ACh) or mechanical stimulation in non-muscle cells, e.g. endothelial cells (Zhou et al. 2005), other epithelial cells (Pitelka & Taggart 1983; Cai & Sheetz 2009) and smooth muscle cells (Osol 1995; Bergner & Sanderson 2002; Cipolla et al. 2002; Mazzetti et al. 2003; Zhang & Gunst 2008). Regulation of contractility in non-muscle cells has been assumed to be similar to the smooth muscle cell paradigm – by events leading eventually to a rise in the cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]_i$) and

the activation of myosin via Ca^{2+}-calmodulin (CaM) mechanism (Goeckeler & Wysolmerski 1995).

Aim of our study was to assess whether similar systems operate also in lens epithelial cells, e.g. in response to ACh stimulation, since in human anterior lens epithelial cells ACh binds to M1 muscarinic receptors and induces a rise in $[Ca^{2+}]_i$ (Collison et al. 2000; Collison & Duncan 2001; Rhodes & Sanderson 2009). We also wanted to assess whether contractions could also occur in response to mechanical stimulation as both mechanisms could be implicated in physiological stress and cataract formation.

Methods

The method is explained in detail in Andjelić et al. 2010. Experiments were done on the anterior lens capsule preparations consisting of the monolayer of epithelial cells attached to the basement membrane, i.e. the capsule matrix. The capsules were obtained routinely during cataract surgery performed at the Eye Hospital, University Medical Centre (UMC), Ljubljana, Slovenia. The research followed the tenets of the Declaration of Helsinki. The study was approved by the National Medical Ethics Committee of the Republic of Slovenia and all patients signed informed consent before the operation. Altogether 101 lens capsules were used. The age of capsule donors was from 48 to 89 with the average being 73 years; 38 were male and 63 female.

Capsule preparation

After the surgery, each capsule was stored in Minimal Essential Medium Eagle (MEM; Sigma-Aldrich, St. Louis, MO, USA), with added heat inactivated newborn calf serum, and transported to the laboratory. The time elapsed from the surgical collection of capsules to ester loading of attached cells was from 10 min to 6 hr 45 min with the average of 2 hr 35 min. Until utilization the capsules were kept in a CO_2 incubator (Innova CO-48; New Brunswick Scientific, Edison, NJ, USA) at 37 °C and 5% CO_2.

The capsules were loaded with the acetoxymethyl (AM) ester of Fura-2 (Fura-2 AM; Invitrogen – Molecular Probes, Carlsbad, CA, USA). For loading Fura-2 AM in DMSO was suspended in 3 ml MEM to a final concentration of 2 μM. The loading was done in the incubator at 37 °C for 30 min. After loading, the capsules were washed twice for 7 min with MEM. Capsules were then transferred to the plastic glass bottom Petri dishes (Mattek Corp., Ashland, MA, USA; 3.5 cm in diameter), filled with 3 ml of the bath solution with (in mM): NaCl 131.8, KCl 5, $MgCl_2$ 2, NaH_2PO_4 0.5, $NaHCO_3$ 2, $CaCl_2$ 1.8, HEPES 10, glucose 10), pH 7.24. There they were immobilized by a harp-like grid, similar to the one used for experiments with small vertebrate brain slices (Konnerth et al. 1987), so that the addition of the agonist solution would not displace them. The grid also flattened the capsule, which was necessary for the optical recording. The orientation of the capsule was with the basement membrane to the bottom, so the agonist could easily diffuse to the cells without having to cross the barrier of the basement membrane. The Petri dish with the immobilized capsule was then mounted on the inverted microscope Zeiss Axiovert S 100 (Carl Zeiss, AG, Oberkochen, Germany).

Stimulus application

In order to evoke responses of epithelial cells we used either global or local applications of liquid stimuli. The latter were done either close (the distance of 450–500 μm horizontally and 450–500 μm vertically) or far (the distance of 1400–1500 μm horizontally and 450–500 μm vertically) from the center of the imaged area. Local far application was used as a control test: the contracting response should not be induced when the puffer pipette used for local liquid stimulation was located far enough from the recording site for the fluid jet to lose its energy. The liquid flow in the local application was controlled by the SP220IZ syringe pump (WPI, Sarasota, FL, USA) in combination with a 3-way solenoid valve [Lee interface fluidic (LIF) series; The Lee Company, Westbrook, CT, USA] connected to a glass pipette mounted on a MM-33 manipulator (Märzhauser, Wetzlar, Germany) while the global application as well as its washout from the bath was driven simply by the hydrostatic pressure of a 35 cm of water column and controlled manually by a luer-lock stopcock (WPI) and applied through a polyethylene plastic tubing (inner diameter 2 mm), attached to the coarse micromanipulator. The excess bathing solution was removed by a suction line. We applied either the agonist acetylcholine (ACh; Sigma, USA) in 10 μM concentration, which was enough to induce >90% maximal $[Ca^{2+}]_i$ response, according to the data by Collison et al. (2000), or the physiological saline alone as a control. To additionally test responses to mechanical stimuli, the mechanical stimulation with a tip of a glass micropipette mounted on a MP-285 micromanipulator (Sutter, Novato, CA, USA) was also used. Prior to use the tip of the pipette was heat-polished until it rounded up.

Image acquisition was done with the 12-bit cooled CCD camera SensiCam (PCO Imaging AG, Kelheim, Germany). The software used for the acquisition was WinFluor (written by J. Dempster, University of Strathclyde, Glasgow, UK). Objectives used were: 40x/0,75 Plan-NeoFluar and 63x/1,25 oil Plan-NeoFluar (Zeiss). The light source used was XBO-75W (Zeiss) Xe arc lamp. The light intensity was attenuated when necessary with grey filters with optical densities 0.5, 1 and 2 (Chroma Technology Corp., Bellows Falls, VT, USA). The excitation filters used, mounted on a Lambda LS-10 filterwheel (Sutter Instruments Co.), were 360 and 380 nm (Chroma). Excitation with the 360 nm filter (close to the Fura-2 isosbestic point) allowed observation of the cells' morphology and of the changes in the concentration of the dye, irrespective of changes in $[Ca^{2+}]_i$, while the 360/380 nm ratio allowed visualization of the $[Ca^{2+}]_i$ changes in the cytoplasm. Image acquisition, timing and filterwheel operation were all controlled by WinFluor software via a PCI6229 interface card (National Instruments, Austin, TX, USA). The criteria for selecting the region for imaging were the presence of adherent cells and good cell morphology both assessed by observation of transilluminated and 360 nm fluorescencent images. Individual image frames were acquired every 500 ms resulting in frame cycles being 1 second long (two wavelengths). All offline analysis was

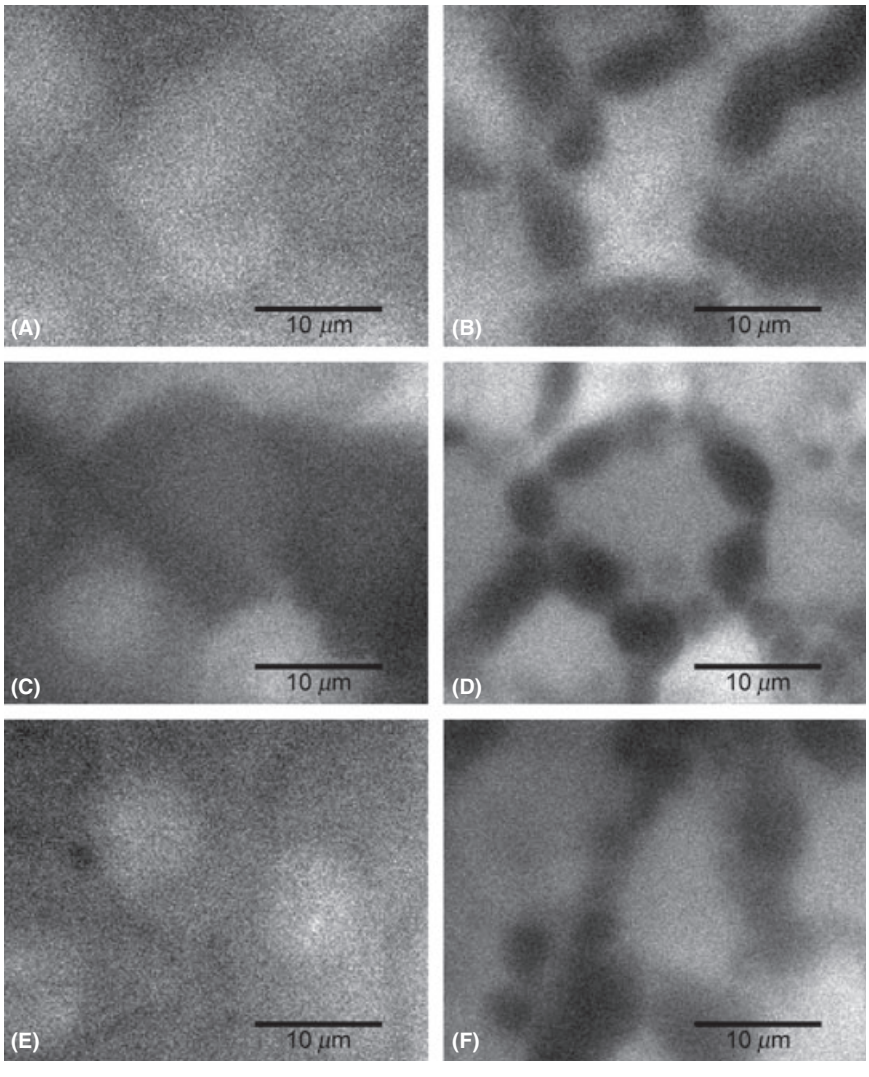

Fig. 1. The contraction of lens epithelial cells. The same frame is shown before (A, C, E) and after the stimulation (B, D, F). Epithelial cells contract in response to several stimuli: global bath application of ACh (B), localized application of physiological solution with a puffer pipette (D) as well as direct local mechanical stimulation with a glass micropipette (F). With frames E and F there is a shift of the scene within the frame by several μm due to the pushing exerted by the stimulation pipette.

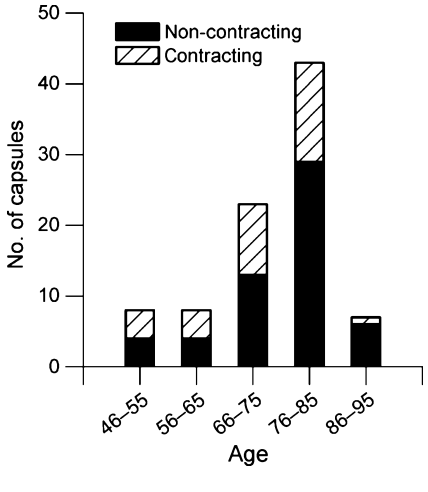

Fig. 2. The distribution of contracting versus non-contracting capsules with the age of the patients.

done in Matlab™ (MathWorks Inc., Natick, CT, USA). The extent of epithelial cell contractions was quantified by calculating the coefficient of variation (CV = the SD to mean ratio) of the 360 nm fluorescence gray values within the selected regions of interest (ROI). Higher values indicate a more unevenly illuminated ROI due to contracted cells with dark areas appearing in between them.

Results

Contractions of lens epithelial cells

An example of a typical contraction evoked by the ACh application (10 μM ACh diluted in the physiological saline) observed at 360 nm excitation, the Fura-2 isosbestic point, is shown on Fig. 1A,B. A contraction is characterized by changes in the shape of individual cells and the appearance of gaps between them. The widths of the gaps varied from hardly noticeable up to several μm across.

Altogether 37% (33/90) of the capsules exhibited the contraction of their epithelial cells. In addition to ACh the use of the physiological saline alone, as well as a direct mechanical stimulus in the form of a rounded tip of a glass micropipette have also caused epithelial cell contractions. In all these cases the visual appearance, as seen with an epifluorescent microscope, was indistinguishable from the case where the agonist application was used (Fig. 1C–F).

The age of the patients whose capsule cells contracted was from 48 to 85 years with the average being 71. We found the contracting behaviour depended neither on age nor gender (Fig. 2) since a similar portion of capsules from both sexes exhibited epithelial cell contractions : 37% (14/38) male and 30% (19/63) female ($p = 0.246$; if not stated otherwise all p values were obtained with the *t*-test).

Table 1 shows the number of preparations with contracting and non-contracting cells depending on time passed between the surgery and the beginning of the experiment. The Pearson correlation coefficient was −0.15, indicating on average slightly lower probability of contractions with longer times passed between the surgery and the experiment. There was however no significant correlation between the time interval and the occurrence of contractions ($p = 0.194$).

To establish whether there was any correlation between the operating surgeon and the percentage of the preparations with contracting cells (Table 2) we used ANOVA. The correlation related to different surgeons-η^2 (the ratio of sum of squares between groups and the total sum of squares) was 0.61, which, however, was not significant ($p = 0.24$; *F*-test).

Contractions induced by the agonist application

When the capsule preparations were stimulated by the application of ACh, cells from all preparations responded

Table 1. Distribution of capsules with contracting and non-contracting cells in time elapsed between the surgery and the beginning of experiment.

Time interval (hr)	*n* contracting	*n* non-contracting	*n* all	% contracting
1–2	5	3	8	62.5
2–3	9	15	24	37.5
3–4	8	27	35	22.8
4–5	2	3	5	40.0
5–6	1	1	2	50.0
6–7	0	1	1	0.0

Table 2. Distribution of capsules with contracting and non-contracting cells among the surgeons who performed the capsulorhexis.

Surgeon	*n* contracting	*n* non-contracting	*n* all	% contracting
1	11	25	36	30.6
2	10	5	15	66.6
3	7	7	14	50.0
4	2	8	10	20.0
5	2	5	7	28.6
6	0	3	3	0.0

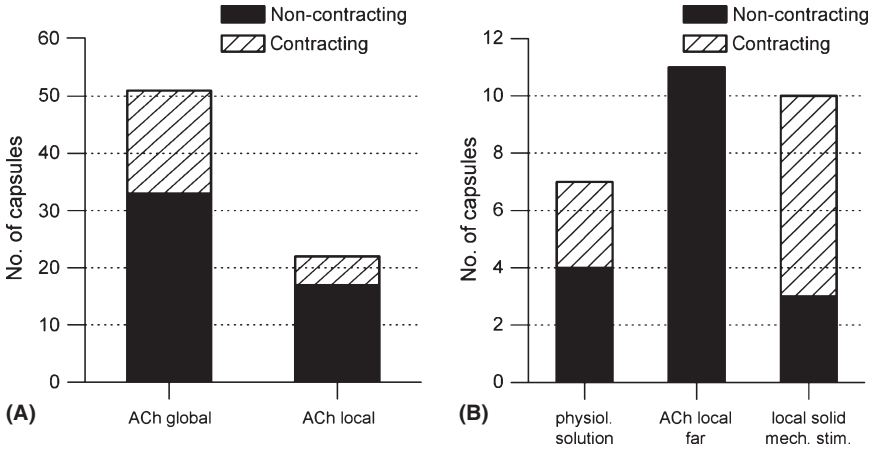

Fig. 3. (A) The proportion of the contracting capsules in the experiments with ACh, global (whole bath) bolus or a local (in close vicinity to the recording site) application with a puffer pipette; B) The proportion of the contracting capsules in the experiments with the physiological solution, local far ACh (far enough from the recording site for the fluid jet to lose its energy) application with a puffer pipette and the solid mechanical stimulation (with a tip of a micropipette).

with an increase in $[Ca^{2+}]_i$, although only a portion – 32% (23/73) of lens capsules exhibited epithelial cell contractions (Fig. 3A). All the observed cells responded to the ACh stimulation with an increase in $[Ca^{2+}]_i$ and no sub-populations with different time courses of changes in $[Ca^{2+}]_i$ could be distinguished. In most cases the rise in $[Ca^{2+}]_i$ was monophasic (Fig. 4D). However, we have also observed more complex responses (e.g. Fig. 4A). Upon ACh stimulation, in preparations where contractions occurred, the 360/380 ratio rose from 1.13 ± 0.02 to 1.35 ± 0.04 and the average rise was 0.22 ± 0.04 (n = 18; all values are in mean ± SEM.).

Two types of ACh applications were used; either a global (whole bath) bolus or a local (in close vicinity to the recording site) application with a puffer pipette. In case of the global application 35% (18/51) of the capsules exhibited contractions of epithelial cells while the percentage was slightly but not significantly lower with local application: 23% (5/22; Fig. 3A; p = 0.148). Figure 5A shows the example of contractions of the anterior lens epithelial cells induced by the global application of ACh and Fig. 5D shows the example of localized contractions following local ACh application. While global application of ACh induced contractions of epithelial cells across the whole preparation (Fig. 5A), local stimulation with ACh, induced local contractions that did not propagate to the whole visual field – only the cells in the region close to the stimulation site were affected by the expelled solution. The cells, still in the visual field, but more distant from the stimulated site, did not contract (Fig. 5D). In the same way a localized application of ACh, far from the recording site, also did not induce any noticeable contractions (Figs 3B and 5C).

Contractions induced without the agonist application

When physiological solution was applied alone, without ACh, cell contractions were also induced in 43% (3/7) of the capsules tested this way (Figs 3B and 5B). In these cases the stimulation evoked changes in $[Ca^{2+}]_i$ were very small (there was hardly any change in $[Ca^{2+}]_i$). The 360/380 ratio changed from 1.25 ± 0.1 to 1.30 ± 0.1 so the actual average rise in the ratio was by 0.05 ± 0.003 (n = 3), which means that the observed rise in $[Ca^{2+}]_i$ could not be attributed to stimulation with certainty while we could still evoke very typical contractions (Fig. 6A–C).

The contractions induced by physiological saline suggested that the mechanical sensitivity may be involved in inducing the contractions of lens epithelial cells. This was confirmed by using direct mechanical stimulation with a polished tip of a micropipette, mounted on a micromanipulator. In this way the small group of cells within the visual field was stimulated. Such stimulation did indeed induce localized contraction in 70% (7/10) of the capsules (Fig. 3B). Although bigger than with physiological saline, the amplitudes of the rise in $[Ca^{2+}]_i$ were smaller than with ACh stimulation. The average evoked rise in the ratio was by 0.17 ± 0.06 (n = 5), compared to the average rise by 0.22 ± 0.04 (n = 22) with ACh. Moreover additional contractions could still be evoked even after $[Ca^{2+}]_i$ began to decay (Fig. 6D–F). Intensity of the

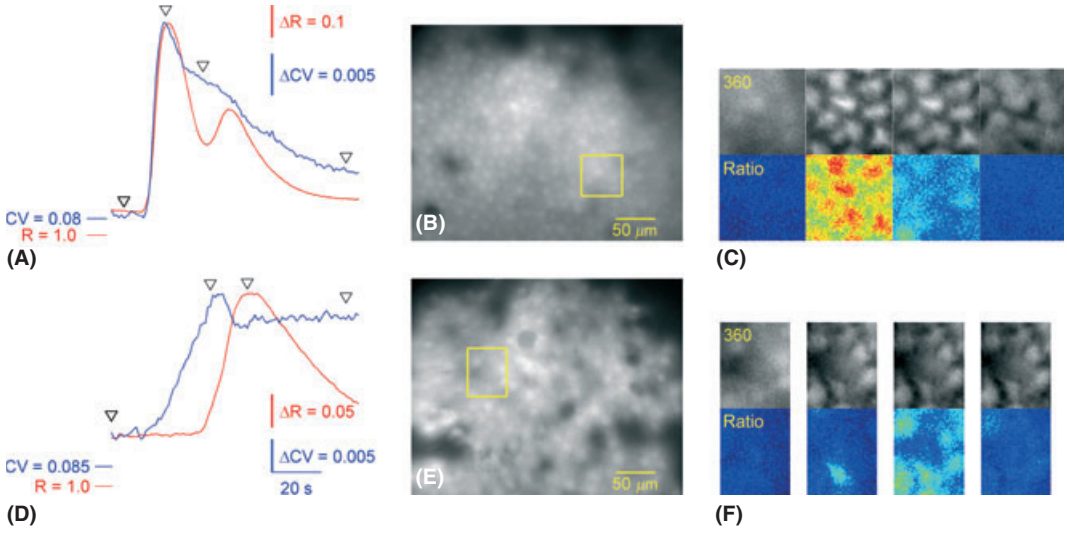

Fig. 4. The contraction of the lens epithelial cells upon ACh stimulation starts either synchronously with the Ca-response (A–C) or before it (D–F). A, D. The time courses of the 360/380 ratio (R), proportional to $[Ca^{2+}]_i$, are shown in red and the time courses of the coefficient of variation, indicating to the changes in morphology, are shown in blue. The two traces correspond to region of interest (ROI) shown on the middle images (B, E). C, F – A series of raw 360 nm fluorescent (top rows) and ratio images (bottom rows) corresponding to the ROI at the time points indicated by triangles on the traces in panels A and D. The values for ratio are colour coded with blue/green representing low ratio values and yellow/red representing high ratios in the ratio values ranging from 1 to 2.

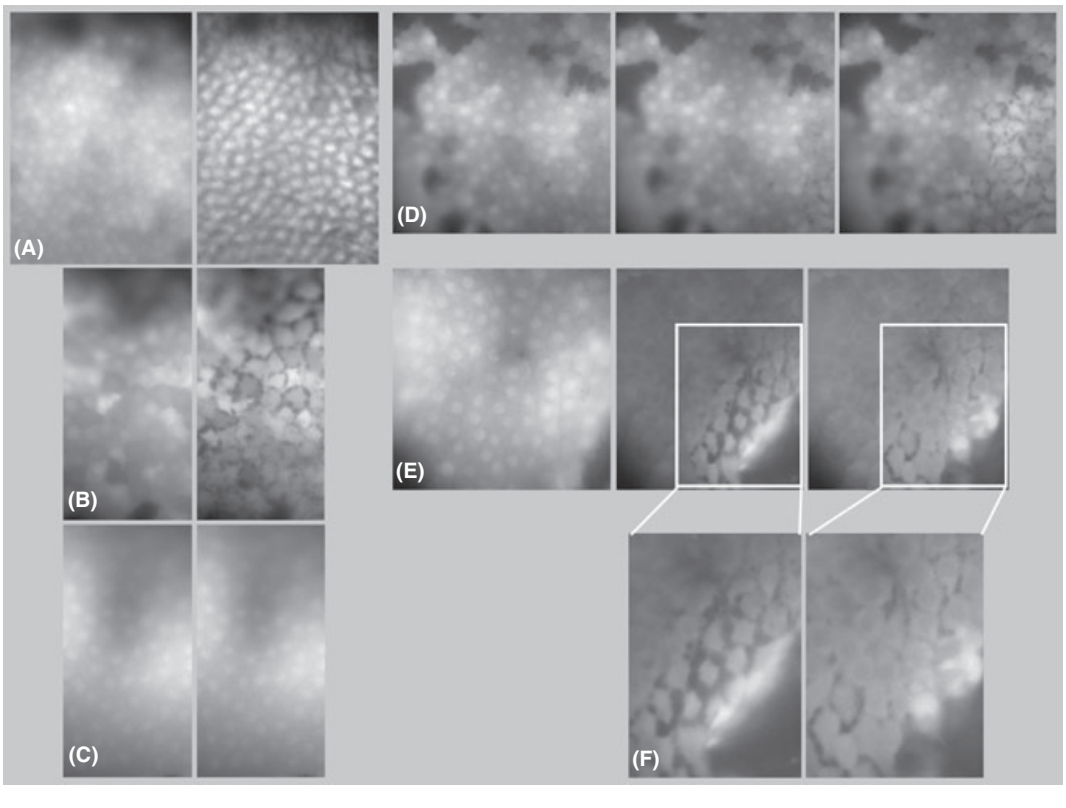

Fig. 5. The contractions of the anterior lens epithelial cells. Global contractions can be induced by global applications of liquid stimuli: acetylcholine (ACh) in the physiological solution (A) and the physiological solution alone (B). Local contractions can be induced by local applications of liquid stimuli as ACh in the physiological solution (D) and local pipette tip mechanical stimulation (E). Contractions are not induced upon local application of liquid stimuli as ACh, far from the recording site (C). The reversibility of cells' contractions is shown on the panel F - the magnified region of panel E.

contraction depended on the intensity of the mechanical stimulation: stronger stimuli evoked stronger contractions of the individual cells and the contractions could be seen over a larger region than was the case with weaker stimuli. Even so the contractions were still localized. Figure 5E shows that the direct local mechanical

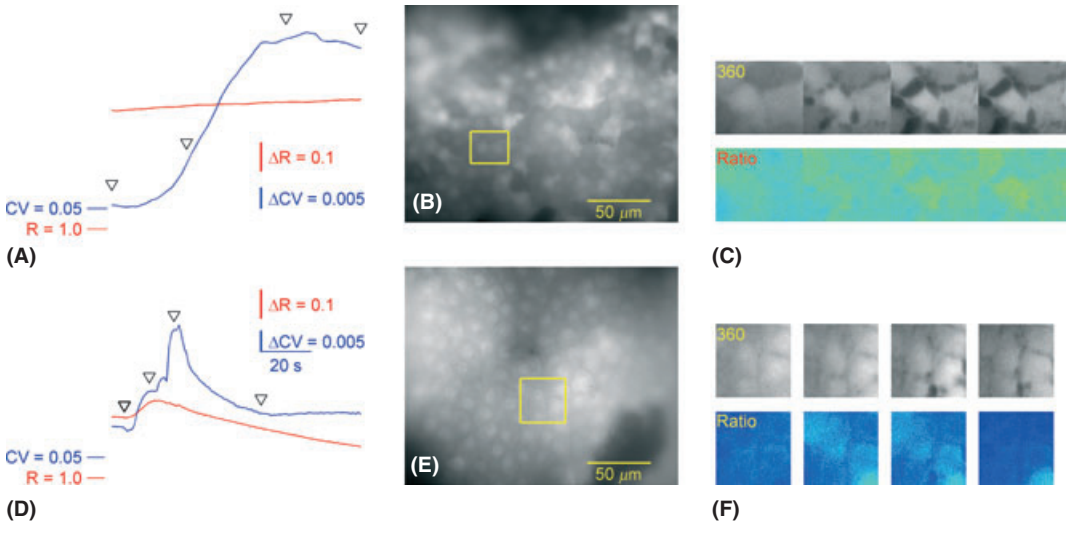

Fig. 6. The contraction occurs both as a response to the fluid jet stimulation by physiological solution (A–C) and to glass micropipette mechanical stimulation (D–F). Yet the intensities of $[Ca^{2+}]_i$ responses are significantly different. A, D. The time course of the 360/380 ratio (R), proportional to $[Ca^{2+}]_i$ is shown in red and the time course of the coefficient of variation, proportional to the changes in morphology is shown in blue. The two traces correspond to regions of interest (ROI) shown on the middle images (B, E). C, F – A series of raw 360 nm fluorescent (top rows) and ratio images (bottom rows) corresponding to the ROI at the time points indicated by triangles on the traces in panels A and D. The values for ratio are colour coded with blue/green representing low ratio values and yellow/red representing high ratios in the ratio values ranging from 1 to 2. Note the color range is the same as in Fig. 4.

stimulation of the group of the cells induced localized contractions of epithelial cells. We also found that the contractions were reversible (Fig. 5F). After the cessation of stimulation, epithelial cells tended to return back to the initial non-contracted state, albeit at a slower rate than they contracted.

Contraction versus cataract type

The cell contractions were observed in all the capsules grouped according the cataract type (Figs 7 and 8). The proportions were: 27% (7/26) of cortical cataract capsules, 35% (12/34) nuclear, and 35% (6/17) of capsules exhibiting a combination of nuclear and cortical cataract. The differences between groups regarding the probability of contractions were not significant, as determined by ANOVA ($p = 0.77$).

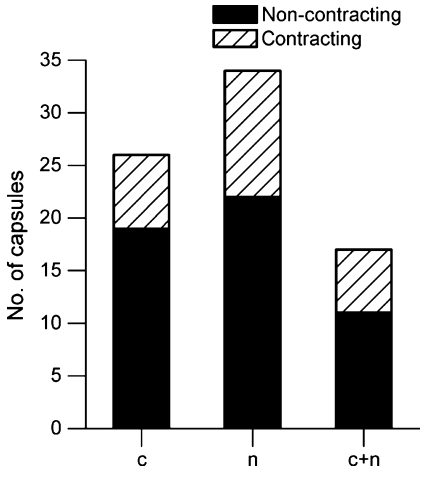

Fig. 7. The distribution of contracting and non-contracting capsules versus cataract type (c – cortical, n – nuclear, c + n – combined cortical and nuclear cataract).

Time parameters of changes in $[Ca^{2+}]_i$ and contractions induced by agonist application

Two typical examples of the time courses of $[Ca^{2+}]_i$ and contractions induced by ACh application are shown in Fig. 4. Interestingly, the contraction never lagged behind the rise in $[Ca^{2+}]_i$ but was at best simultaneous with it. Even more, in 11 out of 15 cases it was actually ahead of the rise in $[Ca^{2+}]_i$. The average time difference between the onset of contraction and the onset of the rise in $[Ca^{2+}]_i$ was 14.1 ± 5.8 seconds ($n = 15$) and there were no significant differences between the capsules obtained from the three different groups of cataracts (ANOVA, $p = 0.72$). An example of a response where cells contracted simultaneously with the Ca-response is shown on panels A–C and an example of the contraction starting before the start of the Ca-response is shown on panels D–F.

Upon ACh application the average time necessary for $[Ca^{2+}]_i$ to reach its peak from the baseline level was 19.6 ± 3.27 seconds in cortical cataract capsules with the amplitude of 0.25 ± 0.063 whereas contractions took on average 23.5 ± 7.49 seconds from the start to maximum ($n = 4$). In nuclear cataract capsules $[Ca^{2+}]_i$ reached its maximum on average in 15.8 ± 3.17 seconds with the amplitude of 0.25 ± 0.12 and contractions in 25.6 ± 10.62 seconds ($n = 5$). For capsules extracted from lenses exhibiting both nuclear and cortical cataracts it took $[Ca^{2+}]_i$ on average 31.7 ± 5.77 seconds to reach its peak with the amplitude of 0.16 ± 0.057 while contractions took 46 ± 12.63 seconds ($n = 6$). The differences between the three groups were marginally significant regarding the time necessary for the rise in $[Ca^{2+}]_i$ (ANOVA; $p = 0.04$), the group standing out being the nuclear + cortical one, and were not significant regarding contractions (ANOVA; $p = 0.25$). The differences in the amplitudes of the rise in $[Ca^{2+}]_i$ among different cataract types were not significant (ANOVA; $p = 0.62$).

The average time necessary for $[Ca^{2+}]_i$ to decay by 50% of its maximal change was 52.72 ± 10.08 seconds ($n = 4$) for cortical cataract, 50.46 ± 7.78 seconds ($n = 5$) for nuclear cataract and 83.89 ± 17.17 seconds

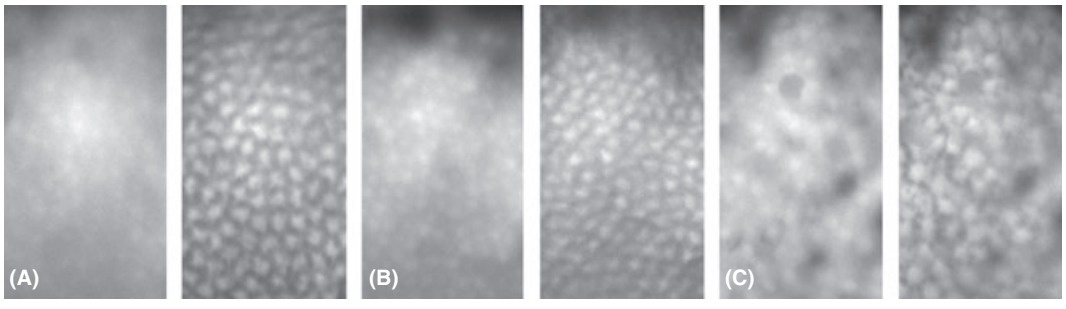

Fig. 8. The contractions of the human anterior lens epithelial cells occur on capsules with (A) cortical, (B) nuclear, (C) cortical + nuclear cataract.

(n = 6) for capsules extracted from lenses exhibiting both nuclear and cortical cataracts. The differences between the three groups were not significant (ANOVA; p = 0.13).

The average time necessary for the cells to return to 50% of their maximally contracted state was 65.67 ± 26.51 seconds (n = 4) for cortical cataract, 72.27 ± 29.11 seconds (n = 5) for nuclear cataract and 121.77 ± 25.49 seconds (n = 6) for capsules extracted from lenses exhibiting both nuclear and cortical cataracts. The differences between the three groups were not significant (ANOVA; p = 0.29).

Since we could find almost no significant differences between cataract types, we could therefore pool all the data and compare the rise times and 50% decay times of $[Ca^{2+}]_i$ and contractions. The average rise time for $[Ca^{2+}]_i$ of 23.16 ± 3.07 seconds was significantly different from the average contraction rise time, which was 33.18 ± 6.37 seconds (p = 0.03, n = 15; one-tailed paired t-test). On the other hand the decay times were much more variable and the difference between $[Ca^{2+}]_i$ and contraction 50% decay times was not significant. $[Ca^{2+}]_i$ decayed by 50% on average in 64.44 ± 8.16 seconds and contractions in 93.09 ± 17.04 seconds (p = 0.08, n = 13[1]; one-tailed paired t-test).

Discussion

In our examination of human lens capsules with the adherent epithelial cells, obtained from routine cataract surgeries, we observed that the epithelial cells contracted in 37% of capsules when stimulated by agonist (ACh) application, fluid movement or direct mechanical stimuli. The occurrence of this phenomenon was unaffected by either age or gender of the donor and did not appear to be correlated with cataract type. Contractions could be induced by the application of the physiological solution and by direct mechanical stimulation. They could not be induced when the puffer pipette used for local liquid stimulation was located far enough from the recording site for the fluid jet to lose its energy. These facts suggest that the contractions are, at least in part, induced mechanically and not by the agonist action of ACh.

Both local liquid and direct mechanical stimulation of capsules, have shown that the contraction occurs locally, at the site of stimulation, and that it does not propagate to the rest of the capsule. The type of contraction of the lens epithelial cells that we were studying is a fast phenomenon that occurs in the range of few seconds rather than days. The reversibility of the contraction suggests that this is an intrinsic property of lens epithelial cells rather than a defect. It also shows that the epithelial cells, attached to the capsules, remain functional and that the contractions are not just a consequence of overstimulation leading to the cell death.

We have tested the possibility that the surgical procedure itself causes this contracting behaviour. We expected that longer times spent in the incubator would, in some way, influence the probability of occurrence of contracting behaviour. However, we found no significant correlations between the occurrence of contractions and the time elapsed from surgical operations as well as between the occurrence of contractions and surgeons performing the operations. Therefore, our present findings do not support the explanation that either mechanical surgical stress or capsule handling directly influences the contracting behaviour of lens epithelial cells.

Although the results obtained from human lens tissue can be directly related to the human lens pathophysiology, no correlations were found between the type of cataract and the occurrence of contractions. Contrary to our expectations there was no significant difference between various types of cataract. Although we have speculated that contractions could be associated with higher occurrence of cortical cataracts, by enlarging the spaces between the cells and short-circuiting the epithelial transport mechanisms, no indications of this actually occurring have been found. This may be viewed as an additional indication that the ability to contract is an intrinsic property of the anterior lens epithelial cells and that it is not necessarily associated with the type of cataract. However, the question whether it may be associated with occurrence of cataract in general cannot be answered satisfactory without the additional information obtained from healthy lens epithelial cells.

The main advantage of the human anterior capsule preparation is, compared to cultures of cells, in preservation of relatively natural conditions. The cells remain connected to neighbouring cells and to the underlying basement membrane. In many types of epithelial cells there is a dense meshwork of F-actin filaments underneath the apical membranes anchored at several points to the apical and lateral membranes. This anchoring is securing the shape of these cells and the intactness of the monolayer. In the lens these apical membranes are

[1]In two experiments the recording was finished before the 50% decay time of contractions was reached.

the fibre facing membranes. The attachment to adjacent cells occurs by adhesion complexes located in the lateral membranes that include both desmosomes and tight junctions (Goodenough 1992; Bhat 2001; Dal & Boulton 2008). During contaction, the cells stay connected to each other at several points, presumably representing regions containing desmosomes and/or gap junctions.

The occurrence of larger and smaller extracellular spaces in cataractous lenses, irrespective of the way cataracts were induced, has been reported previously and named intercellular vacuolization. Most of these reports dealt with extracellular spaces appearing in between the cortical fibre cells (Vrensen et al. 1995; Gao & Spray 1998; Michael et al. 2000), but similar features have been described in lens epithelium as well (Michael et al. 2000; Maddala et al. 2004). At present we have no firm answer whether the phenomenon on which we report is in any way related to this vacuolization, which seems to be related mainly to disturbances in the active ion pumping mechanisms of the lens epithelium. In our experiments there was no visible difference in the epithelial cell morphology prior to stimulation between contracting and non-contracting epithelia. This, in combination with the fact that in our case there did not seem to be any correlation with either cataract type or age, is in itself at odds with previous reports on vacuolization and its relationship to cataracts (Hayes & Fisher 1979; Vrensen et al. 1990; Jongebloed et al. 1998; Hales et al. 1999). However, to properly elucidate this issue more comparative work would be necessary.

Based on our findings, it is currently difficult to determine the actual mechanism behind the observed phenomenon of contractions. Nevertheless, there are some important clues. Even though the cytoskeleton of lens epithelial cells includes actin and myosin (Yeh et al. 1986; Rafferty et al. 1990), both of which are known to be involved in Ca^{2+}-triggered contractions in other cell types, we can, with some confidence, say that $[Ca^{2+}]_i$ is not directly involved in the changes of the shape of epithelial cells described here. However, antagonist studies will be needed to conclusively confirm this observation. In all observed cases, the contraction started either before or at best simultaneously with the rise in $[Ca^{2+}]_i$. Contraction also occurred when there was hardly any change in $[Ca^{2+}]_i$ upon application of physiological solution alone. We can also discard osmotic effects as the direct cause of cell shrinkage, since all the solutions used for stimulation were prepared from the same batch as the bathing solution. An additional confirmation that the mechanism does not involve osmotic effects is the direct mechanical stimulation with a pipette tip, which induced local contractions the appearance of which was indistinguishable from the ones induced by stimulation with an agonist or a fluid jet.

Previously mechanical stimulations have been used to induce a rise in $[Ca^{2+}]_i$ in cultured bovine lens epithelial cells (Churchill et al. 1996). Such stimulation of a single cell within a confluent layer was shown to initiate cell-to-cell calcium signalling without inducing contractions. In our experiments there was one important difference compared to the work of Churchill et al. (1996), apart from the fact that we used human material. The cells we used were not cultured and made to grow to confluence but were rather still attached to the freshly isolated lens capsule. In our experiments mechanical stimulation induced contractions of lens epithelial cells with the intensity of contraction reflecting the intensity of stimulation: stronger the stimulation, stronger the contraction. From this it would seem that the attachment of cells to the capsule basement membrane prior to stimulation is a pre-requisite for the observed contractions. Therefore caution is advisable when discussing possible osmotic effects. It may be that an indirect mechanism, involving only localized osmotic changes between the epithelium and the basement membrane, may still be the cause of the observed epithelial cell contraction or in such case cell shrinkage.

Regardless of whether direct or indirect (osmotic) mechanical action causes the contraction, the possible mechanisms might be associated with mechanosensitive channels. An obvious future goal is to study the family of transient receptor potential ionic channels (Liedtke & Kim 2005; O'Neil & Heller 2005; Thomas et al. 2005; Venkatachalam & Montell 2007; Hoffmann et al. 2009) that may be present in lens epithelial cells since they are present in most other cells comprising the vertebrate eye (Leonelli et al. 2009; Morgans et al. 2009). They are also involved in the pathogenesis of eye diseases like glaucoma (Sappington et al. 2009) and the Lowe syndrome (which includes the lens cataract) (Nilius et al. 2008).

The final question is the functional significance of this phenomenon. The diameter of the human lens epithelial cells ranges from 9 to 17 μm (Brown & Bron 1987) so the contraction of several μm represents significant reduction of the size of these cells. If this actually does occur *in situ*, then gaps forming in between the epithelial cells would very likely seriously impair the normal function of the lens epithelium, which normally provides the driving force for the ionic gradients and the fluid circulation within the lens (Mathias et al. 1997; Zampighi et al. 2000; Candia 2004). The described contractile properties of the anterior lens epithelial cells may possibly also play a role in the lens opacities after exposure to a mechanical trauma or to increased intraocular pressure, for example in cataract after acute glaucoma attacks.

The functional significance of contractions is not clear but the fact that capsule exhibits functional ACh M1 receptor system and elaborate contraction response to mechanical stimulation is interesting. The contractions, which preceded the Ca-responses, were an unexpected finding. In further studies, we therefore plan the use of pharmacological manipulations of M1 receptors and the evaluation of the role of internal calcium stores. Further studies will be also needed to assess the functional significance of the response and its relationship to previously described phenomenon of vacuolization.

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