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Alterations of Calcium Homeostasis Affect the Survival of Human Retinal Epithelial Cells

Chi Kong Yeung,[1,†,*] Kwok-Ping Chan,[1] Sylvia W. Y. Chiang,[1] Chi Pui Pang,[1] Wing-Hung Ko,[2] and Dennis S. C. Lam[1]

[1]Department of Ophthalmology and Visual Sciences, [2]Department of Physiology, The Chinese University of Hong Kong, Kowloon, Hong Kong, People's Republic of China

ABSTRACT

1. This study demonstrated that the modification of calcium concentrations ($[Ca^{2+}]$) could severely affect the viability of human retinal pigment epithelial (ARPE19) cells. 2. Pharmacological agents with varying mechanisms of action: verapamil (VP), diltiazem (DIL), caffeine (CF), papaverine (PA), forskolin (FSK), ryanodine (RYN), thapsigargin (THG), and cyclosporin A (CysA) were used to evaluate the effect of modifying cytosolic Ca^{2+} on the viability of ARPE19 cells. The difference in cell proliferations under different treatments was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Ca^{2+}-imaging was used to determine the changes in intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) over 5 min of drug treatments. 3. Amongst the agents tested, PA, RYN, and THG actually increased the total number of cells initially on Day 1. However, apart from PA, all caused significant reduction of cell viability by Day 5. Variable $[Ca^{2+}]_i$ levels in the cells were obtained with significant rises in the presence of DIL, CF, PA, FSK, and CysA.

†Present address: Bioengineering Graduate Program, Department of Chemical Engineering, Room 2004 A, Academic Building, Clear Water Bay, Kowloon, Hong Kong; Fax: +852-3106-6857; E-mail: ckyeung@ust.hk.

*Correspondence: Dr. Chi Kong Yeung, Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, 3/F., Hong Kong Eye Hospital, 147K Argyle St., Kowloon, Hong Kong; Fax: +852-2715-9490; E-mail: ckcyeung@cuhk.edu.hk.

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Verapamil, RYN, and THG caused a rise in $[Ca^{2+}]_i$, but the effect was not significant. 4. The present study showed that the APRE19 cells were very sensitive to the Ca^{2+} homeostasis. It is possible that other ocular cells may also display such vulnerability. Therefore, clinically used pharmacological agents that are known to affect Ca^{2+} must be treated with caution.

Key Words: Calcium; Cell viability; Cytotoxicity; Retinal pigment epithelial cells.

INTRODUCTION

Calcium (Ca^{2+}) homeostasis plays an important role in the normal physiology and viability of cells (1). The activation of caspase 3 (2), which can be used as an indicator of apoptosis, and its connection with Ca^{2+} have been extensively reviewed (3,4). Thus far there is ample evidence that support the initial Ca^{2+} overload (5–9) and an ultimate increase in caspase activity (5,8–14) as key candidates in apoptosis in a number of cell types.

Other factors that are also important in apoptosis are mitochondrial membrane depolarization and cytochrome c release (5,711,12,14–17), activations of adenylate and guanylate cyclases (18), and activation of inositol triphosphate (IP_3) (19,20). However, these mechanisms all involved Ca^{2+} within the apoptotic pathway, suggesting that the manipulation of which could pose significant changes to the viability of cells.

The manipulations of membrane Ca^{2+} channels (L-type calcium channels), the intracellular endo/sarcoplasmic Ca^{2+} channels (ryanodine receptors mediate Ca^{2+} induced Ca^{2+} release), and up or down regulations of second messengers may reduce or enhance apoptosis-related stress expressions. Indeed, there have been studies that demonstrate the involvement of L-type Ca^{2+} channels in photoreceptor apoptosis (21–24). Calcium channel blockers, verapamil, and diltiazem (21,24,25) have been used to halt or control apoptosis. Approaches to better understanding of the control of apoptosis in the eye by controlling the rise in Ca^{2+} as well as other means have been reviewed (26). It is clear that Ca^{2+} plays a pivotal role in ocular health.

The present study aimed to demonstrate that the human pigment epithelial cells, ARPE19 cells, were sensitive to the changes in Ca^{2+} homeostasis with the use of drugs that are known to affect the Ca^{2+} levels via a variety of mechanisms. Furthermore, we aimed to show that the changes of Ca^{2+} could be responsible for ocular cell death.

MATERIALS AND METHODS

Cell Culture

Human retinal pigment epithelial cells (ARPE19) were purchased from American Type Culture Collection (ATCC, Manassas, VA). The ARPE19 is a spontaneously arising retinal pigment epithelial cell line derived from the normal eyes of a 19-year-old male who died from head trauma. These cells were subjected to selective trypsinization for the first four passages to remove superficial cells before passaging the cuboidal basal layer. By passage 5, the cultures appeared to be rapidly growing retinal pigment epithelial cells, which would form cobblestone monolayers (27). Cell culture

66 reagents, fetal bovine serum, and chemicals were purchased from GIBCO (Rockville,
67 MD), and containers were purchased from Corning (Acton, MA). Human ARPE19
68 cells, within 17–21 passages from the time of purchase, were maintained in 1:1
69 mixture of Dulbecco's modified Eagle's and HAMS F12 medium containing 3 mM
70 L-glutamine supplemented with 10% fetal bovine serum and antibiotic mixtures of
71 100 units/mL penicillin G and 100 μg/mL streptomycin sulphate. Cell suspensions with
72 a cell volume of 5000 cells/mL were seeded onto 24-well tissue culture plastics. After
73 overnight incubation at 37°C and in an environment containing 95% O_2/5% CO_2, the
74 seeded cells were washed gently with phosphate buffered saline to remove cell debris
75 before fresh culture medium was reintroduced. These cells were reincubated in fresh
76 culture medium for at least 1 hour before use.

77 The Pharmacological Study

78 A number of pharmacological agents, all within the known physiologically active
79 concentrations (see below), were used to determine the effect of altering Ca^{2+} levels on
80 cell viability. These drugs were chosen as they target different Ca^{2+} sites within the cell.

81 Calcium Channel Blockers

82 Ca^{2+} channel blocker, verapamil (VP, 1 μM), and diltiazem (DIL, 1 μM) were used
83 to target Ca^{2+} pores directly and thus reduce Ca^{2+} entry. Sterile distilled water was
84 used to make the stocks of both VP and DIL. Subsequent dilutions were made with
85 culture medium.

86 Elevation of Cyclic Adenosine Monophosphate

87 The level of cyclic adenosine monophosphate (cAMP) was increased by using
88 phosphodiesterase inhibitors (papaverine, PA, 10 μM; caffeine, CF, 1 mM) or by
89 activating the adenylate cyclase (forskolin, FSK, 10 μM). Caffeine (1,3,7-trimethylxan-
90 thine) also affects Ca^{2+} levels by depleting Ca^{2+} from intracellular stores. Both PA and
91 CF were made by dissolving directly in culture medium, and the stock of FSK was made
92 with dimethylsulfoxide (DMSO). Subsequent dilutions were made with culture medium.

93 Direct Acting on the Intracellular Ca^{2+} Store Receptors

94 Ryanodine (RYN, 5 μM) was used to directly regulate the intracellular Ca^{2+} levels
95 via receptor-operated channels on the endoplasmic reticulum. Thapsigargin (THG,
96 5 μM) was used as an IP_3-independent intracellular Ca^{2+} releaser and induced
97 mitochondrial permeability transition. Both RYN and THG were made with DMSO and
98 subsequent dilutions with culture medium.

99 Mitochondrial Permeability Transition Pore Inhibitor

100 Cyclosporin A (CysA, 5 μM) was used to inhibit the mitochondrial membrane.
101 The stock of CysA was made with absolute ethanol and subsequent dilutions with
102 culture medium.

The experimental design consisted of two groups: normal growth in the absence of any drug (control group) and the growth of cells in the presence of one of the above agents. These single concentrations of the above agents were determined from the available literature. These concentrations were believed to be physiologically active.

Corresponding drugs at the concentrations stated above were added into the medium of the ARPE19 cell cultures (Day 0). After 24 hours (Day 1), these cells were washed and the amount of cell proliferation was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were washed with phosphate buffered saline (PBS). The MTT at 0.5 mg/mL in serum-free medium was added to the culture and incubated for 3 hours. Formazan extraction was performed using isopropanol and the quantity determined colorimetrically using NanoDrop™ at $\lambda = 570$ nm with the correction of interference at 690 nm in triplicate and five individual samples per group. The effects of these drugs on the viability of the ARPE19 cells and proliferation were also determined on Day 5 after the initial exposure. Appropriate vehicle controls were also performed. Vehicles of the equivalent concentrations of the drugs used were added at the same time as the drugs themselves. These vehicles include (0.01% or 0.1%) ethanol and (0.2% or 0.5%) DMSO.

Calcium Measurements

Intracellular Ca^{2+} ($[Ca^{2+}]_i$) changes were measured using calcium-sensitive fluorescent dye, Fura-2. Cells were grown for 2 days on coverslips before being incubated for 30 min in medium containing the lipophilic, acetymethylester (AM) form of the dye. Coverslips bearing Fura-2-loaded cells were then mounted in a heated chamber (Warner Instrument, Hamden, CT) attached to the stage of an inverted microscope (Nikon TE300, Tokyo, Japan). The cells were superfused with Krebs-Henseleit (KH) solution containing (in mM) NaCl 117, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4, $NaHCO_3$ 24.8, $CaCl_2$ 2.56, D-glucose 11.1 and then continuously bubbled with 95% O_2/5% CO_2 to maintain the pH at 7.4. The various drugs were then added into the chamber and mixed by simple diffusion. Fura-2 fluorescence ratios (excitation wavelength 340 nm and 380 nm; emission wavelength > 510 nm) were recorded from the average of five to eight cells at 1 Hz (PTI Ratio-Master fluorescence system, Photon Technology International, Lawrenceville, NJ, USA). This allows changes in $[Ca^{2+}]_i$ to be monitored using standard, microspectrofluorimetric techniques. Increase in $[Ca^{2+}]_i$ was quantified by measuring the fluorescence ratio at the peak of a response and subtracting from it the ratio measured before stimulation. For the calcium-imaging study, the excitation light source was provided by a multiwavelength illuminator (Polychrome IV, TILL Photonics, Gräfelfing, Germany). The emitted fluorescence was collected using a digital cooled CCD camera (Quantix, Photometrics, Tucson, AZ, USA). Images were digitized and analyzed using MetaFluor V.6.0 (Universal Imaging Corporation, Downingtown, PA, USA).

Measurements and Data Analysis

The results were obtained as units of absorbance of MTT at 570 nm ± SD. However, as the cells were not originated from a single pool of ARPE19, the change in absorbance was converted to a percentage of growth at a particular time point and in a

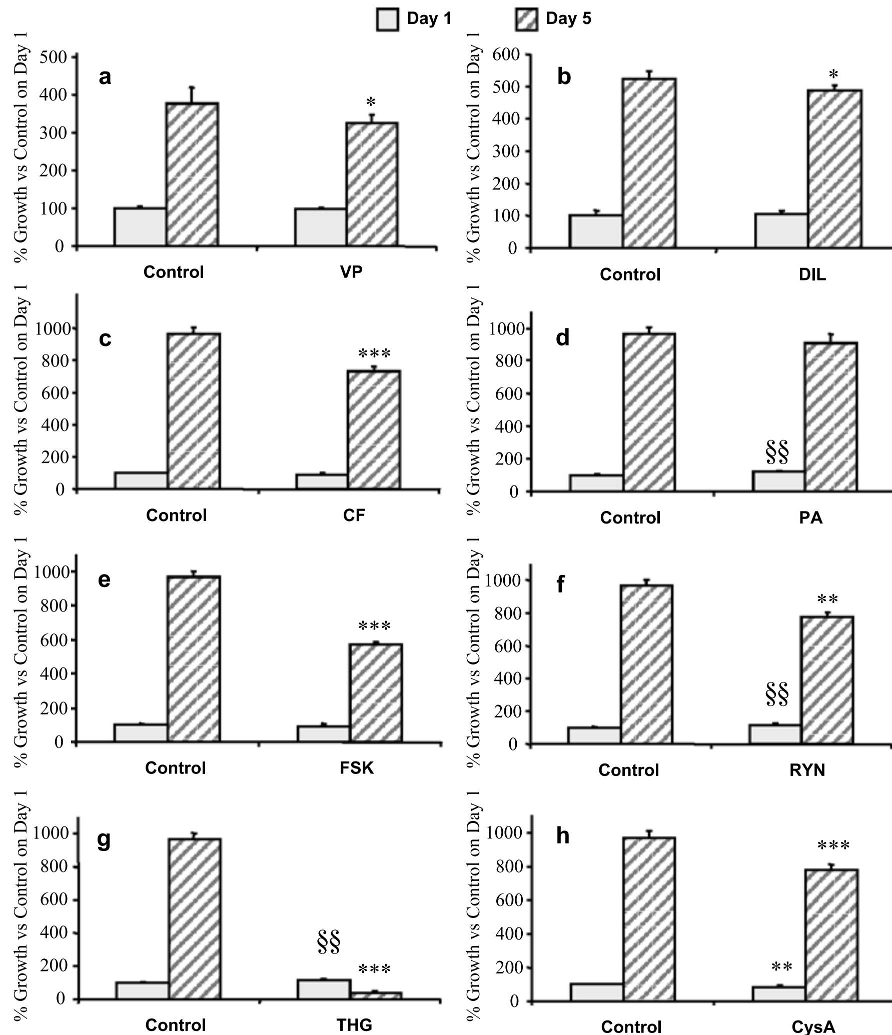

Figure 1. The percentage cell growth of APRE19 in the absence or presence of VP (a), DIL (b), CF (c), PA (d), FSK (e), RYN (f), THG (g), and CysA (h) on Day 1 (filled) and Day 5 (stripes). As the cells did not originate from a single pool of ARPE19, the change in absorbance was converted to a percentage of growth at a particular time point and in a particular condition by dividing the MTT absorbance of cells under the treatment of a drug by the absorbance obtained on Day 1 under control normal growth (i.e., absorbance in the absence or presence of a drug on a particular measured time point÷absorbance of the control on Day 1 × 100%). As such, the absorbance reading of the cells on Day 1 was taken as 100% in all cases. The results generally show that alteration of Ca^{2+} homeostasis can severely reduce the viability of cells. It appeared that changes to cellular physiology with PA, RYN, and THG for the short term actually enhanced cell proliferations. The results are expressed as % ± SD of change in growth vs. that observed on Day 1, which is considered to be 100%. The results were analyzed using ANOVA and n = 5 in all. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; significant reduction in cell number compared with the corresponding control. $^{§§}P < 0.01$; significant increase in cell number compared with the corresponding control.) (*View this art in color at www.dekker.com.*)

146 particular condition by dividing the MTT absorbance of cells under the treatment of a
147 drug by the absorbance obtained on Day 1 under control normal growth (i.e.,
148 absorbance in the absence or presence of a drug on a particular measured time
149 point÷absorbance of the control on Day 1 × 100%). As such, the absorbance reading
150 of the cells on Day 1 was taken as 100% in all cases. The relative difference between
151 controls and drug-treated groups were analyzed using analysis of variance (ANOVA).
152 In calcium-imaging experiments, the changes in intensity were plotted against the
153 average Fura-2 ratios ± SD of five or more cells. The Fura-2 ratio at 0 min was taken
154 as the background $[Ca^{2+}]_i$ before the addition of drugs. Fura 2 ratios at 0 min and
155 ≥ 1 min (i.e., after the drug has been added) were compared using Student's t-test.

156 # RESULTS

157 ## Viability Assay

158 The present study demonstrated that changes in different aspects of Ca^{2+} levels
159 could have a profound effect on the viability of the ARPE19 cells. These changes were
160 brought about by the use of drugs that affect Ca^{2+} via different mechanisms.

161 ## Ca^{2+} Channel Blockers

162 Verapamil (1 μM) and DIL (1 μM) had no effect on cell viability on Day 1, but
163 significant reductions of cells treated with these drugs were seen by Day 5 (Fig. 1a
164 and 1b), which meant that a relatively long period of Ca^{2+} channel blockade might
165 be harmful to cells.

166 ## Elevation of cAMP

167 Both CF (1 mM, Fig. 1c) and FSK (10 μM, Fig. 1e) caused significant reductions
168 in viability of cells on Day 5. Although the result of PA (Fig. 1d) was not significant,
169 there was a small reduction in cell number on Day 5. Papaverine seemed to be able to
170 promote cell growth if the cells were only being exposed to it for one day.

171 ## Drugs that Act on Ca^{2+} Stores

172 Ryanodine (5 μM, Fig. 1f) at this concentration can block Ca^{2+} release from stores.
173 There was a promotion of cell proliferation on Day 1 followed by a significant
174 reduction in the viability of cells on Day 5. Thapsigargin (5 μM, Fig. 1g), an IP_3-
175 independent intracellular Ca^{2+} releaser, has the same profile of effects as RYN.

176 ## Mitochondrial Permeability Transition Pore Inhibitor

177 Cyclosporin A (5 μM, Fig. 1h) inhibited the membrane permeability of the
178 mitochondria. Significant reductions were detected on both Day 1 and Day 5.

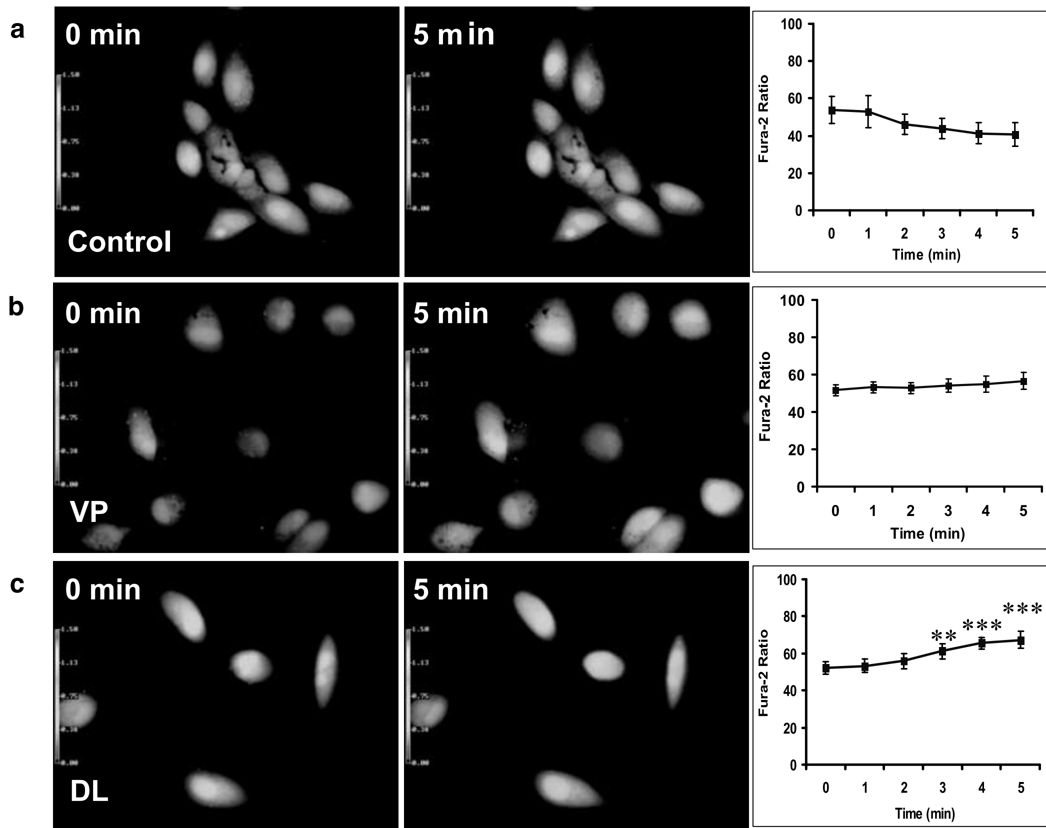

Figure 2. Five-min continuous Fura-2 ratio measurements of an average of five ARPE19 cells (a) exposed to VP (b), DIL (c), CF (d), PA (e), FSK (f), RYN (g), THG (h), and CysA (i). Ca^{2+} images at 0 min and 5 min are shown. The continuous Fura-2 ratio was also given to illustrate the progressive changes upon the presence of different drugs over this 5-min period. The drug was added at 1 min of recording. During this recorded period, the $[Ca^{2+}]_i$ was affected by the presence of the above agents to variable degrees. Within this short period of detection, changes of the $[Ca^{2+}]_i$ were the most rapid with DIL, CF, PA, FSK, and CysA. (*View this art in color at www.dekker.com.*)

179 **Calcium Measurements**

180 The Fura-2 ratio, given by the relative difference between 340/380 nm
181 fluorescence, was used to reflect the change in the $[Ca^{2+}]_i$ (Fig. 2). Compared with
182 their corresponding control measurements taken at 0 min, DIL (Fig. 2c), CF (Fig. 2d),
183 PA (Fig. 2e), FSK (Fig. 2f), and CysA (Fig. 2i) significantly increased $[Ca^{2+}]_i$.
184 However, compared with the normal $[Ca^{2+}]_i$ fluctuation observed in Fig. 2a, there was
185 a general rise in $[Ca^{2+}]_i$. Although this change in $[Ca^{2+}]_i$ was not significant with VP
186 (Fig. 2b), RYN (Fig. 2g), and THG (Fig. 2h), it does show that there is a general
187 upward trend in the $[Ca^{2+}]_i$. In certain cases, the extent of Ca^{2+} changes of these agents

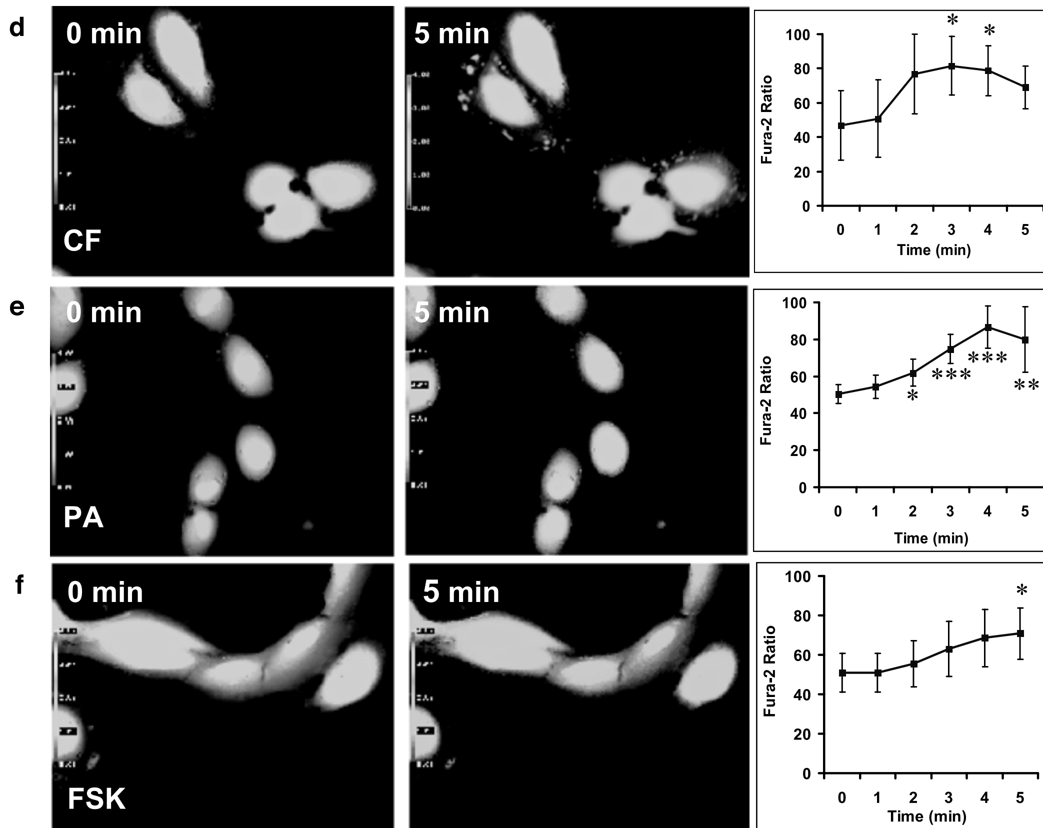

Figure 2. Five-min continuous Fura-2 ratio measurements of an average of five ARPE19 cells (a) exposed to VP (b), DIL (c), CF (d), PA (e), FSK (f), RYN (g), THG (h), and CysA (i). Ca^{2+} images at 0 min and 5 min are shown. The continuous Fura-2 ratio was also given to illustrate the progressive changes upon the presence of different drugs over this 5-min period. The drug was added at 1 min of recording. During this recorded period, the $[Ca^{2+}]_i$ was affected by the presence of the above agents to variable degrees. Within this short period of detection, changes of the $[Ca^{2+}]_i$ were the most rapid with DIL, CF, PA, FSK, and CysA. (*Continued*).

188 reflects the viability results showing that the $[Ca^{2+}]_i$ of different groups was indeed
189 altered within this short space of time.

190 DISCUSSION

191 The influence of Ca^{2+} on cellular physiology is complex. Among many processes,
192 the Ca^{2+} levels can be affected by the property of membrane Ca^{2+} channels,
193 intracellular second messengers, calcium stores, and the related Ca^{2+}-induced Ca^{2+}
194 release with a number of drugs. The present study used a variety of agents that are

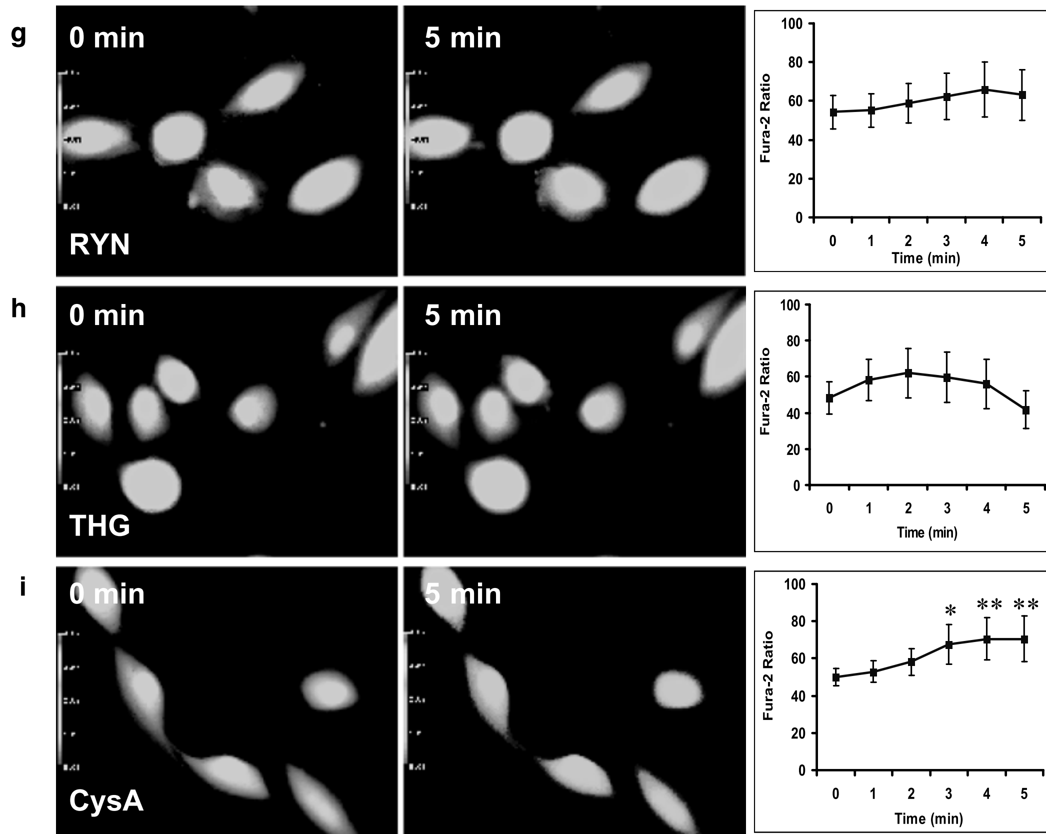

Figure 2. (*Continued*).

195 known to affect $[Ca^{2+}]_i$ via different mechanisms of action, showing that the alteration
196 of the physiological $[Ca^{2+}]_i$ can have a profound effect on the viability of cells.
197 When Ca^{2+} channel blockers alone were in contact with the APRE19 cells by
198 the end of Day 5, the number of viable cells remaining reduced. It is possible that
199 the prolonged reduction and continued absence of Ca^{2+} entry can adversely affect the
200 energy production of cells leading to more unnecessary stress. Indeed, there is evidence
201 showing that the reduction of cytosolic Ca^{2+} can affect the mitochondrial function,
202 and this disruption of mitochondrial Ca^{2+} homeostasis can lead to cellular toxicity
203 (15,16,28–30).
204 Similarly, the increase in intracellular cAMP concentration with CF (a
205 phosphodiesterase inhibitor/deplete calcium) and FSK (activation of adenylate cyclase)
206 caused reductions in cell numbers by Day 5. Papaverine (a phosphodiesterase inhibitor)
207 actually increased the number of cells on Day 1. The mere fact that CF is a Ca^{2+}
208 releaser/decalcifier may be enough to exacerbate the extent of necrosis of the ARPE19
209 cells by elevating the cytosolic Ca^{2+} concentration momentarily. It is well known that
210 the activation of cAMP will reduce Ca^{2+} influx across the plasma membrane or inhibit

211 Ca^{2+} release from Ca^{2+} stores. Further, inhibition of phosphodiesterase, which
212 hydrolyzes cAMP, will cause the accumulation of cAMP (31). Papaverine, when used
213 alone, showed cellular protective ability on Day 1, which might have been due to its
214 Ca^{2+} blocking ability as well as reduced Ca^{2+} overload. Although it seems that
215 reducing Ca^{2+} will improve viability, it is very much dependent on the duration of
216 exposure much like what was observed with VP and DIL.
217 Ryanodine and THG at the concentrations used have been shown to activate the
218 ryanodine receptor and trigger an IP_3-independent Ca^{2+} mobilization from stores,
219 respectively (32,33). The toxic mechanism of RYN is mainly due to mitochondrial
220 swelling (34) and the function of THG is based on activating the mitochondrial
221 permeability transition and releasing Ca^{2+} from the mitochondrial matrix Ca^{2+}-binding
222 sites (35,36). The use of these drugs on the ARPE19 cells simply substantiated the fact
223 that excess cytosolic Ca^{2+} in cells could increase necrosis or osmotic lysis and the
224 importance of mitochondrial functions in cellular health.
225 Cyclosparin A, which acts by inhibiting the mitochondrial permeability transition
226 pore, has recently been found to be safe and useful in a number of ocular ailments such
227 as superior limbic keratoconjunctivitis and pediatric keratoplasty (37,38). Although
228 CysA has been shown to have a protective effect against Ca^{2+}-induced apoptosis of the
229 rod photoreceptor (6), it has also been shown that CysA may contribute to the
230 development of steroid-induced cataract (39). The present study clearly indicated that
231 CysA has a negative effect on cell viability. Since the available evidence suggests that
232 CysA could be beneficial as well as toxic, clinical use must be viewed with caution.

233 CONCLUSIONS

234 It seems clear that modification of Ca^{2+} directly with Ca^{2+} channel blockers or
235 indirectly by affecting the Ca^{2+} stores, second messenger systems, or other intracellular
236 structures can affect the physiological state of the cell. The results suggest that changes
237 in $[Ca^{2+}]_i$ via a number of mechanisms can cause cell necrosis. The data in the present
238 study simply imply that the presence of these chosen agents can severely affect the
239 viability of cells, and this in turn reduces the number of cell proliferations per unit
240 time. This shows that retinal pigment epithelial cells are very sensitive to the changes
241 of Ca^{2+} homeostasis, judging by the viability assay and the elevated $[Ca^{2+}]_i$, further
242 illustrating that $[Ca^{2+}]_i$ is responsible for eventual cell necrosis. In the eye, a number of
243 pharmacological agents are used to treat a variety of ocular ailments. Considering that
244 the retinal epithelial cells in this study show such a sensitive change to their viability,
245 clinically used pharmacological agents that are known to affect Ca^{2+} must be treated
246 with caution. As found with RYN and THG, even agents that demonstrate growth
247 promotional effect at an early stage of administration may well cause severe
248 cytotoxicity at a later stage following prolonged use.

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252 REFERENCES

253 1. Riccardi D, Gamba G. The many roles of the calcium-sensing receptor in health and
254 disease. Arch Med Res 1999; 30(6):436–448.
255 2. He L, Perkins GA, Poblenz AT, Harris JB, Hung M, Ellisman MH, Fox DA. Bcl-xL
256 overexpression blocks bax-mediated mitochondrial contact site formation and
257 apoptosis in rod photoreceptors of lead-exposed mice. Proc Natl Acad Sci 2003;
258 100(3):1022–1027.
259 3. Linden R, Rehen SK, Chiarini LB. Apoptosis in developing retinal tissue. Prog
260 Retin Eye Res 1999; 18(2):133–165.
261 4. Hajnoczky G, Davies E, Madesh M. Calcium signalling and apoptosis. Biochem
262 Biophys Res Commun 2003; 304(3):445–454.
263 5. Fox DA, He L, Poblenz AT, Medrano CJ, Blocker YS, Srivastava D. Lead-
264 induced alterations in retinal cGMP phosphodiesterase trigger calcium overload,
265 mitochondrial dysfunction and rod receptor apoptosis. Toxicol Lett 1998; 102–
266 103:359–361.
267 6. Fox DA, Poblenz AT, He L, Harris JB, Medrano CJ. Pharmacological strategies to
268 block rod photoreceptor apoptosis caused by calcium overload: a mechanistic
269 target-site approach to neuroprotection. Eur J Ophthalmol 2003; Suppl. 3:S44–S56.
270 7. He L, Poblenz AT, Medrano CJ, Fox DA. Lead and calcium produce rod receptor
271 cell apoptosis by opening the mitochondrial permeability transition pore. J Biol
272 Chem 2000; 275(16):12175–12184.
273 8. Sur P, Sribnick EA, Wingrave JM, Nowak MW, Ray SK, Banik NL. Estrogen
274 attenuates oxidative stress-induced apoptosis in C6 glial cells. Brain Res 2003;
275 971(2):178–188.
276 9. Miwa K, Nakamura J, Hamada Y, Naruse K, Nakashima E, Kato K, Kasuya Y,
277 Yasuda Y, Kamiya H, Hotta N. The role of polyol pathway in glucose-induced
278 apoptosis of cultured retinal pericytes. Diabetes Res Clin Pract 2003; 60(1):1–9.
279 10. Cellerino A, Bahr M, Isenmann S. Apoptosis in the developing visual system. Cell
280 Tissue Res 2000; 301(1):53–69.
281 11. Annuniziato L, Amoroso S, Pannaccione A, Cataldi M, Pignataro G, D'Alessio A,
282 Sirabella R, Secondo A, Sibaud L, Di Renzo GF. Apoptosis induced in neuronal
283 cells by oxidative stress: role played by caspases and intracellular calcium ions.
284 Toxicol Lett 2003; 139(2–3):123–133.
285 12. Mattson MP, Liu D. Mitochondrial potassium channels and uncoupling proteins in
286 synaptic plasticity and neuronal cell death. Biochem Biophys Res Commun 2003;
287 304(3):539–549.
288 13. He Q, Montalbano J, Corcoran C, Jin W, Huang Y, Sheikh MS. Effect of
289 Bax deficiency on death receptor 5 and mitochondrial pathways during endo-
290 plasmic reticulum calcium pool depletion-induced apoptosis. Oncogene 2003;
291 22(17):2674–2679.
292 14. Shiraga S, Adamus G. Mechanism of CAR syndrome: anti-recoverin antibodies are
293 the inducers of retinal cell apoptotic death via caspase 9- and caspase 3-dependent
294 pathway. J Neuroimmunol 2002; 132(1–2):72–82.
295 15. Blomgren K, Zhu C, Hallin U, Hagberg H. Mitochondria and ischemic reperfusion
296 damage in the adult and in the developing brain. Biochem Biophys Commun 2003;
297 304(3):551–559.

298 16. Nieminen AI. Apoptosis and necrosis in health and disease: role of mitochondria.
299 Int Rev Cytol 2003; 224:29–55.
300 17. Brackenridge DG, Stojanvoic M, Marcellus RC, Shore GC. Caspase cleavage
301 product of BAP31 induces mitochondrial fission through endoplasmic reticulum
302 calcium signals, enhancing cytochrome c release to the cytosol. J Cell Biol 2003;
303 160(7):1115–1127.
304 18. Donovan M, Carmody RJ, Cotter TG. Light-induced photoreceptor apoptosis in
305 vivo requires neuronal nitric-oxide synthase and guanylate cyclase activity and is
306 caspase-3-independent. J Biol Chem 2001; 276(25):23000–23008.
307 19. Hajnoczky G, Csorda G, Madesh M, Pacher P. Control of apoptosis by Ipx and
308 ryanodine receptor driven calcium signals. Cell Calcium 2000; 28:349–363.
309 20. Toborek M, Chalimoniuk M. Macrophage migration inhibitor factor (MIF) can
310 induce oxidative injury and apoptotic cell death of spinal cord neurons. J
311 Neurochem 2003; 85(Suppl. 2):29.
312 21. Donovan M, Cotter TG. Caspase-independent photoreceptor apoptosis in vivo and
313 differential expression of apoptosis protease activating factor-1 and caspase-3
314 during retinal development. Cell Death Differ 2002; 9(11):1220–1231.
315 22. Read DS, McCall MA, Gregg RG. Absence of voltage-dependent calcium
316 channels delays photoreceptor degeneration in rd mice. Exp Eye Res 2002;
317 75(4):415–420.
318 23. Yagami T, Ueda K, Asakura K, Nakazato H, Kuroda T, Sakaeda T, Sakaguchi G,
319 Itoh N, Hashimoto Y, Hori Y. Human group IIA secretory phospholipase A_2
320 potentiates Ca^{2+} influx through L-type voltage-sensitive Ca^{2+} channels in cultured
321 rat cortical neurons. J Neurochem 2003; 85(3):749–758.
322 24. Hart J, Wilkinson MF, Kelly ME, Barnes S. Inhibitory action of diltiazem on
323 voltage-gated calcium channels in cone photoreceptors. Exp Eye Res 2003;
324 76(5):597–604.
325 25. Tsutsui S, Itagaki S, Kawamura S, Harada K, Karaki H, Doi K, Yoshikawa Y.
326 D-galactosamine induced hepatocyte apoptosis is inhibited in vivo and in cell
327 culture by a calcium calmodulin antagonist, chlorpromazine, and a calcium channel
328 blocker, verapamil. Exp Anim 2003; 52(1):43–52.
329 26. Khokhar S, Chawla R. Apoptosis: an ocular perspective. Natl Med J India 2002;
330 15(3):148–153.
331 27. Dunn KC, Aotaki-Keen AE, Putkey FR, Hjelmeland LM. ARPE19, a human retinal
332 pigment epithelial cell line with differentiated properties. Exp Eye Res 1996;
333 62:155–169.
334 28. Rolo AP, Oliveira PJ, Seica R, Santos MS, Moreno AJ, Palmeira CM. Disruption of
335 mitochondrial calcium homeostasis after chronic alpha-naphthylisothiocyanate
336 administration: relevance for cholestasis. J Investig Med 2002; 50(3):193–200.
337 29. Heng JE, Vorwerk CK, Lessell E, Zurakowski D, Levin LA, Dreyer EB.
338 Ethambutol is toxic to retinal ganglion cells via an excitotoxic pathway. Investig
339 Ophthalmol Vis Sci 1999; 40(1):190–196.
340 30. El Idrissi A, Trenkner E. Growth factors and taurine protect against excitotoxicity
341 by stabilizing calcium homeostasis and energy metabolism. J Neurosci 1999;
342 19(21):9459–9468.
343 31. Takagi K, Takayanagi I, Tomiyama A. Actions of dibutyryl cyclic adenosine

344 monophosphate, papaverine, and isoprenaline on intestinal smooth muscle. Jpn J
345 Pharmacol 1971; 21:447–483.
346 32. Abrenica B, Pierce GN, Gilchrist JS. Nucleoplasmic calcium regulation in rabbit
347 aortic vascular smooth muscle cells. Can J Physiol Pharm 2003; 81(3):301–310.
348 33. Lotem K, Gal H, Mama R, Amariglio N, Rechavi G, Domany E, Sachs L, Givol D.
349 Inhibition of p53-induced apoptosis without affecting expressions of p53-regulated
350 genes. Proc Natl Acad Sci 2003; 100(11):6718–6723.
351 34. Mungarro-Menchaca X, Ferra P, Moran J, Arias C. beta-Amyloid peptide induces
352 ultrastructural changes in synaptosomes and potentiates mitochondrial dysfunction
353 in the presence of ryanodine. J Neurosci Res 2002; 68(1):89–96.
354 35. Korge P, Weiss JN. Thapsigargin directly induces the mitochondrial permeability
355 transition. Eur J Biochem 1999; 265(1):273–280.
356 36. Thomas GR, Sanderson J, Duncan G. Thapsigargin inhibits a potassium
357 conductance and stimulates influx in the intact rat lens. J Physiol 1999; 516
358 (Pt 1):191–199.
359 37. Perry HD, Doshi-Carnevale S, Donnenfeld ED, Kornstein HS. Topical cyclosporine
360 A 0.5% as a possible new treatment for superior limbic keratoconjunctivitis.
361 Ophthalmology 2003; 110(8):1578–1581.
362 38. Cosar CB, Laibson PR, Cohen EJ, Rapuano CJ. Topical cyclosporine in pediatric
363 keratoplasty. Eye Contact Lens 2003; 29(2):103–107.
364 39. Nakamura T, Sasaki H, Nagaki K, Fujisawa K, Sasaki K, Suzuki K, Tsugawa R.
365 Influence of cyclosporin on steroid-induced cataracts after renal transplantation. Jpn
366 J Ophthalmol 2003; 47(3):254–259.
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	C/Author first name:	Chi Kong
	C;A/Author middle initial:	
	C:A/Author last name:	Yeung
	A/Suffix to last name:	
	C;A/Degrees:	Ph.D.
	C;A/Author e-mail address:	ckcyeung@cuhk.edu.hk; ckcyeung@ust.hk
	C;A/Author fax:	+ 852-2715-9490; + 852-3106-6857
	C;A/Author phone:	+ 852-2762-3155
Affiliation(s) at time of authorship	C;A/Title	
	C;A/Department	Department of Ophthalmology and Visual Sciences
	C;A/Institution	The Chinese University of Hong Kong
	C;A/Street Address	3/F., Hong Kong Eye Hospital, 147K Argyle Street
	C;A/City	Kowloon
	C;A/State/Province	MISSING DATA S/B QUERIED TO THE CONTACT AUTHOR
	C;A/Postal code	
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Affiliation(s) at time of authorship	C;A/Title	
	C;A/Department	Bioengineering Graduate Program, Department of Chemical Engineering
	C;A/Institution	MISSING DATA S/B QUERIED TO THE CONTACT AUTHOR
	C;A/Street Address	Room 2004 A, Academic Building, Clear Water Bay
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