GABA$_{\alpha 1}$ and GABA$_{\rho 1}$ subunits are expressed in cultured human RPE cells and GABA$_{\alpha}$ receptor agents modify the intracellular calcium concentration

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**Purpose:** Gamma-aminobutyric acid$_{\alpha}$ (GABA$_{\alpha}$) receptors (GABA$_{\alpha}$Rs), which are ionotropic receptors involving chloride channels, have been identified in various neural (e.g., mouse retinal ganglion cells) and nonneural cells (e.g., mouse lens epithelial cells) regulating the intracellular calcium concentration ([Ca$^{2+}$]). GABA$_{\alpha}$Rs are mainly located in the neural system and retina [3,4], but have also been detected in many nonneural cells and tissues, for example, in human peripheral blood mononuclear cells [5], human hepatic cells and carcinomas [6], the human prostate [7], the human thyroid [8], murine enteroendocrine cell line STC-1 [9], cat chemosensory glomus cells [10], and the rat taste bud [11] and kidney [12]. In the eye, GABA$_{\alpha}$Rs are mainly located in the neural system and retina [3,4], but have also been detected in many nonneural cells and tissues, for example, in human peripheral blood mononuclear cells [5], human hepatic cells and carcinomas [6], the human prostate [7], the human thyroid [8], murine enteroendocrine cell line STC-1 [9], cat chemosensory glomus cells [10], and the rat taste bud [11] and kidney [12]. In the eye, GABA$_{\alpha}$Rs are mainly located in the neural system and retina [3,4], but have also been detected in many nonneural cells and tissues, for example, in human peripheral blood mononuclear cells [5], human hepatic cells and carcinomas [6], the human prostate [7], the human thyroid [8], murine enteroendocrine cell line STC-1 [9], cat chemosensory glomus cells [10], and the rat taste bud [11] and kidney [12]. In the eye, GABA$_{\alpha}$Rs are mainly located in the neural system and retina [3,4], but have also been detected in many nonneural cells and tissues, for example, in human peripheral blood mononuclear cells [5], human hepatic cells and carcinomas [6], the human prostate [7], the human thyroid [8], murine enteroendocrine cell line STC-1 [9], cat chemosensory glomus cells [10], and the rat taste bud [11] and kidney [12]. In the eye, GABA$_{\alpha}$Rs are mainly located in the neural system and retina [3,4], but have also been detected in many nonneural cells and tissues, for example, in human peripheral blood mononuclear cells [5], human hepatic cells and carcinomas [6], the human prostate [7], the human thyroid [8], murine enteroendocrine cell line STC-1 [9], cat chemosensory glomus cells [10], and the rat taste bud [11] and kidney [12]. In the eye, GABA$_{\alpha}$Rs are mainly located in the neural system and retina [3,4], but have also been detected in many nonneural cells and tissues, for example, in human peripheral blood mononuclear cells [5], human hepatic cells and carcinomas [6], the human prostate [7], the human thyroid [8], murine enteroendocrine cell line STC-1 [9], cat chemosensory glomus cells [10], and the rat taste bud [11] and kidney [12]. In the eye, GABA$_{\alpha}$Rs are mainly located in the neural system and retina [3,4], but have also been detected in many nonneural cells and tissues, for example, in human peripheral blood mononuclear cells [5], human hepatic cells and carcinomas [6], the human prostate [7], the human thyroid [8], murine enteroendocrine cell line STC-1 [9], cat chemosensory glomus cells [10], and the rat taste bud [11] and kidney [12]. In the eye, GABA$_{\alpha}$Rs have been reported to regulate intracellular calcium concentration ([Ca$^{2+}$]) in a variety of cells. The...
The RPE cell cultures were established from five donor eyecups from one eye of each of five previously healthy adults after the corneas were removed for donor cornea transplantation surgery. Donors included three males (aged 38, 73, and 75 years) and two females (aged 66 and 70 years) of Han nationality. Eyecups were received within 24 h of death. The study was approved by the Institutional Review Board of Shandong University’s Qilu Hospital and was performed in accordance with “The Code of Ethics of the World Medical Association (Declaration of Helsinki)” for experiments involving humans.

Primary cultures of human RPE cells were produced as previously described [40,41]. After the anterior segments and vitreous were removed, the retinal tissue was separated from the RPE and was retained for use as the positive control. After being washed three times in PBS (1X; 120 mM NaCl, 20 mM KCl, 10 mM NaPO_4, 5 mM KPO_4, pH 7.4; Gibco, Rockville, MD), the posterior eyecups were incubated with 0.25% trypsin-EDTA (Gibco) and the RPE cells were collected using Dulbecco’s modified Eagle’s medium (DMEM; manufacturer product number: 11965-092, Gibco, Rockville, MD) with 10% fetal bovine serum (Gibco, Rockville, MD) and seeded in a culture flask; cells from each donor were kept separate (n = 5). When reaching confluence, the cells were digested using 0.25% trypsin-EDTA, and were passaged; the experiment used the third passage cells. The cell phenotype was identified using immunofluorescence with an RPE-specific marker, namely the RPE 65 antibody (Millipore, Billerica, MA) [42]. To determine whether the cultured cells were contaminated by other cells such as glial cells [43], Müller cells [44], fibroblasts [45], or choroidal melanocytes [46], the cultured cells were also stained with S100 antibody (Zhongshan Jinqiao Company, Beijing, China).

**Real-time PCR:** Total RNA extraction and reverse transcription were performed as previously described [16]. Based on the sequences reported in the GenBank database, primers for human GABA_A_α1 and GABA_A_α2 were designed and ordered from Shanghai Biosune Biotechnology Company (Shanghai, China). The primer sequences of human GABA_A_α1 were F: 5’- GGC TGG TAC AAC CGT CAG, R: 5’- CTC CCA ATC CTG GTG TCA GGC GA-3’. The sequences of human GABA_A_α2 were F: 5’- GGC TGG TAC AAC CGT CTC TA-3’, R: 5’- CAC AAA GCT GAC CCA GAG GT-3’. RNA concentration and purity were determined at an optical density ratio of 260:280 using a spectrophotometer. SYBR Green real-time PCR was accomplished according to the manufacturer’s protocol. Briefly, denaturation was performed for 10 s at 95 °C, annealing for 10 s at 60 °C, and extension for 10 s at 72 °C. β-Actin was used as the housekeeping gene. The real-time PCR products were sent to the Shanghai Biosune Biotechnology Company for sequence analysis. Correct product size was confirmed by DNA agarose gel and lack of primer dimer formation was verified by melt curve analysis. The human retina was used as the positive control, and the samples without cDNA were used as the negative control.

**Western blots:** Western blots were performed as previously described [16]. Total protein was extracted separately from each RPE sample and human retina sample (which served as the positive control). Proteins were separated by 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), and transferred to polyvinylidene difluoride (PVDF) membranes. After being blocked in 5% fat-free milk diluted in Tris-buffered saline with Tween (TBST; 0.1% Tween-20, 150 mM NaCl, 50 mM Tris, pH 7.5) for 1 h, the
membrane was incubated with the polyclonal goat anti-human GABA\textsubscript{A\alpha1} antibody (1:100, Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal goat anti-human GABA\textsubscript{A\rho1} antibody (1:100, Santa Cruz, CA), or monoclonal mouse anti-human \(\beta\)-Actin antibody (Zhongshan Jinqiao Company, Beijing, China) overnight at 4 °C, and then was followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibody (1:10,000) for 1 h at 37 °C. The bands developed by use of HRP-conjugated secondary antibody detection kits (Jingmei, Shenzhen, China) were scanned and analyzed with Fluorchem\textsuperscript{TM} 9900 Gel Imaging System (Alpha Innotech, San Leandro, CA).

**Immunofluorescence:** Immunofluorescence was performed as previously described [16,46,47]. Briefly, after being fixed with 4% paraformaldehyde for 15 min, cells were blocked with 10% normal donkey serum for 30 min at room temperature and then were incubated with monoclonal mouse anti-human RPE antibody (1:300), monoclonal mouse anti-human S100 antibody (1:200), polyclonal goat anti-human GABA\textsubscript{A\alpha1} antibody (1:50), and polyclonal goat anti-human GABA\textsubscript{A\rho1} antibody (1:50) overnight at 4 °C. Subsequently, the cells were incubated with donkey anti-mouse secondary antibody (Alexa Fluor 568 conjugated; 1:1,000; Invitrogen, CA) and donkey anti-goat secondary antibody (Alexa Fluor 488 conjugated; 1:1,000; Invitrogen) for 30 min at 37 °C. Cells incubated with PBS instead of primary antibodies served as negative control. A drop of Prolong Gold anti-fade reagent with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen) was added before cell images were acquired using an LSM 710 laser confocal microscope (EC Plan-Neofluar 40×/1.30 Oil objective, N.A. 0.55) equipped with ZEN 2009 Light Edition software (Zeiss, Germany).

**Measuring \([\text{Ca}^{2+}]\text{i}\):** \([\text{Ca}^{2+}]\text{i}\) was measured as previously described [48]; \(\text{Ca}^{2+}\)-dependent RPE functions and the use of RPE cell cultures to study these have been recently reviewed [28]. RPE cells were seeded onto specialized cell culture dishes 35 mm in diameter with a glass disc of 20 mm in diameter inserted in the middle of the base (NEST, Wuxi, China). When cultures were confluent, the cells were incubated with 5 μM fluo3-acetoxymethyl ester (Fluo3-AM) calcium indicator for 20 min in the dark at 37 °C in normal physiological saline solution (N-PSS; 140 mM NaCl, 1 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM glucose, 5 mM HEPES, pH 7.4). After being rinsed twice with N-PSS, cells were kept in N-PSS for another 10 min; the cell culture dishes with RPE attached were placed on the viewing stage of a confocal microscope (LSM 710, Zeiss). While images were being captured, the GABA\textsubscript{A} agonist muscimol (100 μM; Sigma, MO) was added to the assay at set time points. To determine the impact of the GABA\textsubscript{A}R antagonist, cells were preincubated with either the GABA\textsubscript{A}R antagonist picrotoxin (300 μM; Sigma) or the GABA\textsubscript{A\rho1} antagonist TPMPA (500 μM; Sigma) for 10 min before muscimol (100 μM) was added. To determine the effects of muscimol on \([\text{Ca}^{2+}]\text{i}\) in buffer without \(\text{Ca}^{2+}\), cells were rinsed and kept in PSS without \(\text{Ca}^{2+}\) added. PBS was added instead of agents as the control. Sequences of images were acquired using the laser confocal microscope (LSM 710, Zeiss) equipped with a 488 nm laser at 5 s intervals.

The fluorescent intensity over the cultured human RPE cell body was measured before and after agent application, and was calculated and analyzed using Zen 2009 Light Edition software (Zeiss).

**Statistical analysis:** Data were acquired from the five different cell samples and each was repeated at least in duplicate. Numerical data were analyzed using SPSS software (SPSS Inc., version 16.0, Chicago, IL) and were expressed as mean ± standard deviation (SD). The differences in the baseline fluorescent intensity across treatment groups in the buffer either with \(\text{Ca}^{2+}\) or without \(\text{Ca}^{2+}\) were analyzed using one-way ANOVA and the Dunnett’s post-hoc test. The differences of fluorescent intensity between the baseline and that after either muscimol or PBS was added in each group were analyzed using paired t-test. Differences were defined as significant at p<0.05.

**RESULTS**

**Human RPE cell culture and identity:** The cultured primary human RPE cells reached confluence in 2–3 weeks. The third passage cells were all positively stained with the RPE 65 antibody and were negative for the S100 antibody (Figure 1). This suggests that the cultured cells were RPE cells and were not contaminated with other cells such as glial cells [43], Müller cells [44], fibroblasts [43], or choroidal melanocytes [45].

**GABA\textsubscript{del} and GABA\textsubscript{del} mRNA expression in cultured human RPE:** Real-time PCR showed that GABA\textsubscript{A\alpha1} and GABA\textsubscript{A\rho1} mRNAs were detected in cultured human RPE and in the retina (positive control), but not in the negative control. Ethidium bromide–stained agarose gels of real-time PCR products showed specific bands present at the positions of 211 bp (GABA\textsubscript{A\alpha1}), 263 bp (GABA\textsubscript{A\rho1}^−), and 302 bp (\(\beta\)-Actin) in cultured human RPE and retina samples, but not in the negative control (Figure 2). The sequence analysis revealed that the sequence of the products corresponded to the targeted mRNA sequence of the GABA\textsubscript{A\alpha1} and GABA\textsubscript{A\rho1}^−.
**GABA**<sub>α1</sub> and **GABA**<sub>ρ1</sub> protein expression in cultured human RPE: Western blots revealed intense bands at 51 kDa, 48 kDa, and 43 kDa for samples incubated with the anti-GABA<sub>α1</sub>, anti-GABA<sub>ρ1</sub>, and anti-β-Actin antibody, respectively, in cultured human RPE and retinal samples (positive control; Figure 3A).

Immunofluorescence revealed immunoreactivity to antibodies for GABA<sub>α1</sub> and GABA<sub>ρ1</sub> in the cultured human RPE cells but not in the negative control. Immunofluorescence was mainly observed in the cell membrane and was also identified in the cytoplasm, but not in the nucleus (Figure 3B).

**GABA**<sub>R</sub> agents modify the [Ca<sup>2+</sup>]<sub>i</sub> in cultured human RPE: There were no significant differences in baseline fluorescent intensity across treatment groups in the buffer either with Ca<sup>2+</sup> (one-way ANOVA, p>0.05, n=5), or without Ca<sup>2+</sup> (one-way ANOVA, p>0.05, n=5). The GABA<sub>α</sub> R agonist muscimol (100 μM) induced a rapid and significant [Ca<sup>2+</sup>]<sub>i</sub> increase in the cultured human RPE cells in the buffer either with Ca<sup>2+</sup> (paired t-test, p<0.05, n=5; Figure 4A) or without Ca<sup>2+</sup> (paired t-test, p<0.05, n=5; Figure 4D). The [Ca<sup>2+</sup>]<sub>i</sub> reached its peak in 20–40 s, and then gradually declined (Figure 4A,D). When the cultured human RPE cells were preincubated with either the GABA<sub>α</sub> R antagonist picrotoxin (300 μM; Figure 4B,E) or the GABA<sub>ρ1</sub> antagonist TPMPA (500 μM; Figure 4C,F), the muscimol (100 μM) induced [Ca<sup>2+</sup>]<sub>i</sub> increase was completely blocked (both when the buffer contained Ca<sup>2+</sup>, Figure 4B,C, and when it did not, Figure 4E,F). The addition of the control agent, PBS, did not alter the [Ca<sup>2+</sup>]<sub>i</sub> of the cultured human RPE cells (paired t-test, p>0.05, n=5).
DISCUSSION

GABA_{α1} and GABA_{ρ1} mRNAs and proteins were expressed in the cultured human RPE cells; the protein was primarily located in the cell membrane, and was also present in the cytoplasm, but was not observed in the nucleus. The GABA_{A}R agonist muscimol induced a [Ca^{2+}]_{i} rise in the cultured human RPE cells irrespective of whether the buffer contained Ca^{2+} or not, and the [Ca^{2+}]_{i} increase was completely blocked by the GABA_{A}R antagonist picrotoxin and the GABA_{ρ} antagonist TPMPA. This suggests that both GABA_{α1} and GABA_{ρ1} occur in cultured human RPE cells and that in these cells, GABA_{A}R stimulation can modify [Ca^{2+}]_{i}. This means that GABA_{α3} [14], GABA_{β} and GABA_{δ} [38], and now GABA_{α1} and GABA_{ρ1} have been identified in cultured human RPE cells, collectively showing that human RPE cells possess various GABA receptors, and thus that the GABAergic pathways in the RPE are likely complex.

In the retina, the GABA_{A}R agonist muscimol increase the [Ca^{2+}]_{i} in the early embryonic chick retina [49] and in postnatal mouse retinal ganglion cells [26]. The GABA_{A}R
antagonist picrotoxin has been shown to attenuate the muscimol-induced [Ca\textsuperscript{2+}] increase in postnatal mouse retinal ganglion cells [26]. In this study, we observed that the GABA\textsubscript{\alpha}R agonist muscimol increase the [Ca\textsuperscript{2+}] in cultured human RPE cells and that the antagonist picrotoxin blocked this rise. This suggests that activation of the GABA\textsubscript{\alpha}R modifies the [Ca\textsuperscript{2+}] in RPE cells as it does in retinal ganglion cells [26].

In the retina, the GABA\textsubscript{\alpha} antagonist TPMPA has been shown to suppress both the GABA-induced current and the light-evoked feedback inhibition observed in ON-cone bipolar cells and to enhance the light-evoked excitatory postsynaptic currents of ON-transient amacrine cells [50]. TPMPA reduces the stimulation thresholds of ON-center retinal ganglion cells [51], and increases the light responsiveness of retinal ganglion cells in a rat model of retinitis pigmentosa [52]. Here, we found that TPMPA inhibited the muscimol induced [Ca\textsuperscript{2+}] increase in cultured human RPE cells; suggesting that the GABA\textsubscript{\alpha} receptor functions are not unique to the retina.

GABA\textsubscript{\alpha} receptors have been shown to be involved in eye growth and myopia development in animal models [53-58], and levels of GABA transporter 1 (GAT-1) increased in the myopic mouse retina [59]. The GABA\textsubscript{\alpha} receptor agonist muscimol induced myopia development in chicks [53] and prevented the myopia-reducing effects of normal vision [54], whereas the GABA\textsubscript{\alpha} antagonist TPMPA inhibited form-deprivation myopia in both chicks [53,54] and guinea pigs [56,58]. The pathway, targets, and mechanisms for the eye growth effects of these GABA agents are unclear. Previously, as GABA\textsubscript{\alpha} receptors are expressed in retina [4], and the GABA\textsubscript{\alpha}R agonist muscimol and the GABA\textsubscript{\alpha} antagonist TPMPA modify the functions of many retinal cells [26,50,51], the retina was considered the most plausible target. In this study, we found that GABA\textsubscript{\alpha} and GABA\textsubscript{\alpha} are expressed in the human RPE, and that muscimol and TPMPA modulate the [Ca\textsuperscript{2+}]. Thus, the RPE is a potential additional site for the actions of muscimol and TPMPA. The sclera ultimately determines ocular size [60], and thus, how activation of the GABA\textsubscript{\alpha} receptors in the RPE might influence scleral physiology requires further investigation.

Additional roles of GABA receptors and their impacts on RPE physiology require investigation. Here, we have shown that GABA agents can modify the [Ca\textsuperscript{2+}] of RPE cells. Ca\textsuperscript{2+} acts as a second messenger controlling many cellular processes, including secretion, cell differentiation, and signal transmission (reviewed in [61]). For example, in cultures of RPE cells, calcium antagonists have been shown to reduce RPE cell proliferation and increase pigmentation.
The Ca$^{2+}$ balance is critical for preventing the accumulation of lipofuscin in RPE cells during phagocytosis [31]. [Ca$^{2+}$]i overload causes lipofuscin accumulation, and this can be prevented by a Ca$^{2+}$ antagonist that suppresses Ca$^{2+}$ influx [31]. In cultured mouse lens epithelial cells [15] GABA agents can modify the [Ca$^{2+}$]i, and the authors [15] speculate that GABA-mediated Ca$^{2+}$ signaling may be used to prevent sustained Ca$^{2+}$ overload, which can both cause cataract (reviewed in [63]) and trigger apoptosis [64]. In the RPE, GABA-mediated Ca$^{2+}$ signaling is thus likely to have similarly critical cellular roles. In summary, GABA$\alpha_{1}$ and GABA$_{\alpha P}$ mRNAs and proteins were expressed in cultured human RPE cells, and the GABA$_{R}$ agonists muscimol, antagonist picrotoxin, and GABA$_{\alpha P}$ antagonist TPMPA were shown to modify [Ca$^{2+}$]i.

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REFERENCES

1. Chebib M, Johnston GA. GABA-Activated ligand gated ion channels: medicinal chemistry and molecular biology. J Med Chem 2000; 43:1427-47. [PMID: 10780899].

2. Olsen RW, Sieghart W. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol Rev 2008; 60:243-60. [PMID: 18790874].

3. Olsen RW, Sieghart W. GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 2009; 56:141-8. [PMID: 18760291].

4. Yang XL. Characterization of receptors for glutamate and GABA in retinal neurons. Prog Neurobiol 2004; 73:127-50. [PMID: 15201037].

5. Alam S, Laughton DL, Walding A, Wolstenholme AJ. Human peripheral blood mononuclear cells express GABA$\alpha$ receptor subunits. Mol Immunol 2006; 43:1432-42. [PMID: 16213022].

6. Bautista W, Perez-Alvarez V, Burczynski F, Raouf A, Klonisch T, Minuk G. Membrane potential differences and GABA$\alpha$ receptor expression in hepatic tumor and non-tumor stem cells. Can J Physiol Pharmacol 2014; 92:85-91. [PMID: 24383877].

7. Abdul M, McCray SD, Hoosien NM. Expression of gamma-aminobutyric acid receptor (subtype A) in prostate cancer. Acta Oncol 2008; 47:1546-50. [PMID: 18607852].

8. Roberts SS, Mendonca-Torres MC, Jensen K, Francis GL, Vasko V. GABA receptor expression in benign and malignant thyroid tumors. Pathol Oncol Res 2009; 15:645-50. [PMID: 19381877].

9. Glassmeier G, Herzig KH, Hopfner M, Lemmer K, Jansen A, Scherubl H. Expression of functional GABA$\alpha$ receptors in cholecystokinin-secreting gut neuroendocrine murine STC-1 cells. J Physiol 1998; 510:805-14. [PMID: 9660895].

10. Igarashi A, Zadzika N, Shirahata M. Benzodiazepines and GABA-GABAA receptor system in the cat carotid body. J Physiol 1998; 510:805-14. [PMID: 9660895].

11. Cao Y, Zhao FL, Kolli T, Hivle R, Herness S. GABA expression in the mammalian taste bud functions as a route of inhibitory cell-to-cell communication. Proc Natl Acad Sci USA 2009; 106:4006-11. [PMID: 19223578].

12. Takano K, Yatabe MS, Abe A, Suzuki Y, Sanada H, Watanabe T, Kimura J, Yatabe J. Characteristic expressions of GABA receptors and GABA producing/transporting molecules in rat kidney. PLoS ONE 2014; 9:e105835-. [PMID: 25188493].

13. Seigel GM, Sun W, Salvi R, Campbell LM, Sullivan S, Reidy JJ. Human corneal stem cells display functional neuronal properties. Mol Vis 2003; 9:159-63. [PMID: 12724646].

14. Wood JP, Osborne NN. Immunological localization of GABA$\alpha$-receptor subunits in cultured human and rat retinal pigment epithelium. Exp Eye Res 1996; 63:113-6. [PMID: 8983956].

15. Schwirtlich M, Kwakowsky A, Emri Z, Antal K, Lacza Z, Cselenyay A, Katarova Z, Szabó G. GABAergic signaling in primary lens epithelial and lentoid cells and its involvement in intracellular Ca$^{2+}$ modulation. Cell Calcium 2011; 50:381-92. [PMID: 21820173].

16. Cheng ZY, Wang XP, Schmid KL, Liu L. Identification of GABA receptors in chick retinal pigment epithelium. Neurosci Lett 2013; 539:43-7. [PMID: 2384571].

17. Cheng ZY, Chebib M, Schmid KL. rh1 GABAC receptors are expressed in fibrous and cartilaginous layers of chick sclera and located on sclera fibroblasts and chondrocytes. J Neurochem 2011; 118:281-7. [PMID: 21554320].

18. Nilsson M, Eriksson PS, Ronnback L, Hansson E. GABA induces Ca$^{2+}$ transients in astrocyte. Neuroscience 1993; 54:605-14. [PMID: 8332251].

19. Owens DF, Boyce LH, Davis MB, Kriegstein AR. Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by garamicidin perforated-patch recordings and calcium imaging. J Neurosci 1996; 16:6414-23. [PMID: 8815920].

20. Li YX, Schaffner AE, Walton MK, Barker JL. Astrocytes regulate developmental changes in the chloride ion gradient of embryonic rat ventral spinal cord neurons in culture. J Physiol 1998; 509:847-58. [PMID: 9596804].
21. Ikeda Y, Nishiyama N, Saito H, Katsuki H. Furosemide-sensitive calcium rise induced by GABAA-receptor stimulation in cultures of embryonic rat striatal neurons. Jpn J Pharmacol 1997; 74:165-9. [PMID: 9243324].

22. Eilers J, Plant TD, Marandi N, Konnerth A. GABA-mediated Ca2+ signalling in developing rat cerebellar Purkinje neurons. J Physiol 2001; 536:429-37. [PMID: 11600678].

23. Lorsignol A, Taupignon A, Dufy B. Long-term GABA receptor activation increases [Ca2+]i in single lactotrophs. Am J Physiol 1996; 270:E793-801. [PMID: 8967467].

24. Spergel DJ, Krsmamovic LZ, Stojiljkovic SS, Catt KJ. L-type Ca2+ channels mediate joint modulation by gamma-aminobutyric acid and glutamate of [Ca2+]i and neupeptide secretion in immortalized gonadodropin-releasing hormone neurons. Neuroendocrinology 1995; 61:499-508. [PMID: 7617127].

25. Williams B, Bence M, Everest H, Forrest-Owen W, Lightman SL, McArdi CL. GABAA receptor mediated elevation of Ca2+ and modulation of gonadotropin-releasing hormone action in alphaF3–1 gonadotropes. J Neuroendocrinol 2000; 12:159-66. [PMID: 10718911].

26. Dayanithi G, Chen-Kuo-Chang M, Viero C, Hamel C, Muller A, Lenaers G. Characterization of Ca2+ signalling in post-natal mouse retinal ganglion cells: involvement of OPA1 in Ca2+ clearance. Ophthalmic Genet 2010; 31:53-65. [PMID: 20450306].

27. Bonilha VL. Retinal pigment epithelium (RPE) cytoskeleton in vivo and in vitro. Exp Eye Res 2014; 126:38-45. [PMID: 24090540].

28. Reichhart N, Strauss O. Ion channels and transporters of the retinal pigment epithelium. Exp Eye Res 2014; 126:27-37. [PMID: 25152360].

29. Rosenthal R, Heimann H, Agostini H, Martin G, Hansen LL, Strauss O. Ca2+ channels in retinal pigment epithelial cells regulate vascular endothelial growth factor secretion rates in health and disease. Mol Vis 2007; 13:443-56. [PMID: 17417605].

30. Wimmers S, Strauss O. Basal calcium entry in retinal pigment epithelial cells is mediated by TRPC channels. Invest Ophthalmol Vis Sci 2007; 48:5767-72. [PMID: 18055830].

31. Zhang L, Hui YN, Wang YS, Ma JX, Wang JB, Ma LN. Calcium overload is associated with lipofuscin formation in human retinal pigment epithelial cells fed with photoreceptor outer segments. Eye (Lond) 2011; 25:519-27. [PMID: 21311572].

32. Stalman P, Himpen B. A decreased Ca2+-wave propagation is found among cultured RPE cells from dystrophic RCS rats. Invest Ophthalmol Vis Sci 1998; 39:1493-502. [PMID: 9660499].

33. Yang P, Baci P, Parker Kerrigan B, Etheridge M, Sung E, Toimil BA, Berchuck JE, Jaffe GJ. Retinal Pigment Epithelial Cell Death by the Alternative Complement Cascade: Role of Membrane Regulatory Proteins, Calcium, PKC and Oxidative Stress. Invest Ophthalmol Vis Sci 2014; 55:3012-21. [PMID: 24677108].

34. Friedman Z, Hackett SF, Campochiaro PA. Human retinal pigment epithelial cells possess muscarinic receptors coupled to calcium mobilization. Brain Res 1988; 446:11-6. [PMID: 3370475].

35. Maneu V, Gerona G, Fernandez L, Cuenca N, Lax P. Evidence of alpha 7 nicotinic acetylcholine receptor expression in retinal pigment epithelial cells. Vis Neurosci 2010; 27:139-47. [PMID: 20932358].

36. Moroi-Fetters SE, Earley O, Hirakata A, Caron MG, Jaffe GJ. Binding, coupling, and mRNA subtype heterogeneity of alpha 1-adrenergic receptors in cultured human RPE. Exp Eye Res 1995; 60:527-32. [PMID: 7615018].

37. Rymer J, Miller SS, Edelman JL. Epinephrine-induced increases in [Ca2+]i and KCl-coupled fluid absorption in bovine RPE. Invest Ophthalmol Vis Sci 2001; 42:1921-9. [PMID: 11431462].

38. Cheng ZY, Wang XP, Schmid KL, Han XG. GABA and GABA receptor subunits co-expressed in cultured human RPE cells regulate intracellular Ca via G-protein and phospholipase C pathways. Neuroscience 2014; 280C:254-61.

39. Ragazzino D, Woodward RM, Murata Y, Eusebi F, Overman LE, Miledi R. Design and in vitro pharmacology of a selective gamma-aminobutyric acidC receptor antagonist. Mol Pharmacol 1996; 50:1024-30. [PMID: 8863850].

40. Chen R, Hollborn M, Grosche A, Reichenbach A, Wiedemann P, Bringmann A, Kohen L. Effects of the vegetable polyphenols epigallocatechin-3-gallate, luteolin, apigenin, myricetin, quercetin, and cyanidin in primary cultures of human retinal pigment epithelial cells. Mol Vis 2014; 20:242-58. [PMID: 24623967].

41. Cao S, Walker GB, Wang X, Cui JZ, Matsubara JA. Altered cytokine profiles of human retinal pigment epithelium: oxidant injury and replicative senescence. Mol Vis 2013; 19:718-28. [PMID: 23598666].

42. Hamel CP, Tsilou E, Pfeffer BA, Hooks JJ, Detrick B, Redmond TM. Molecular cloning and expression of RPE65, a novel retinal pigment epithelium-specific microsomal protein that is post-transcriptionally regulated in vitro. J Biol Chem 1993; 268:15751-7. [PMID: 8340400].

43. Vinores SA, Herman MM, Hackett SF, Campochiaro PA. A morphological and immunohistochemical study of human retinal pigment epithelial cells, retinal glia, and fibroblasts grown on Gelfoam matrix in an organ culture system. A comparison of structural and nonstructural proteins and their application to cell type identification. Graefes Arch Clin Exp Ophthalmol 1993; 231:279-88. [PMID: 8319918].

44. Terenghi G, Cocchia D, Michetti F, Dian AR, Peterson T, Cole DF, Bloom SR, Polak JM. Localization of S100 protein in Muller cells of the retina–1. Light microscopical immunocytochemistry. Invest Ophthalmol Vis Sci 1983; 24:976-80. [PMID: 6345449].
45. Valtink M, Engelmann K. Serum-free cultivation of adult normal human choroidal melanocytes. Graefes Arch Clin Exp Ophthalmol 2007; 245:1487-94. [PMID: 17458555].

46. Economopoulou M, Hammer J, Wang F, Fariss R, Maminishkis A, Miller SS. Expression, localization, and function of junctional adhesion molecule-C (JAM-C) in human retinal pigment epithelium. Invest Ophthalmol Vis Sci 2009; 50:1454-63. [PMID: 19060272].

47. Juel HB, Faber C, Svendsen SG, Vallejo AN, Nissen MH. Inflammatory cytokines protect retinal pigment epithelial cells from oxidative stress-induced death. PLoS ONE 2013; 8:e64619. [PMID: 23705001].

48. Ryan JS, Baldridge WH, Kelly ME. Purinergic regulation of cation conductances and intracellular Ca2+ in cultured rat retinal pigment epithelial cells. J Physiol 1999; 520:745-59. [PMID: 10545141].

49. Yamashita M, Fukuda Y. Calcium channels and GABA receptors in the early embryonic chick retina. J Neurobiol 1993; 24:1600-14. [PMID: 8301268].

50. Matsui K, Hasegawa J, Tachibana M. Modulation of excitatory synaptic transmission by GABA(C) receptor-mediated feedback in the mouse inner retina. J Neurophysiol 2001; 86:2285-98. [PMID: 11698519].

51. Jensen RJ, Rizzo JF 3rd. Effects of GABA receptor antagonists on thresholds of P23H rat retinal ganglion cells to electrical stimulation of the retina. J Neural Eng 2011; 8:035002. [PMID: 21593547].

52. Jensen RJ. Blocking GABA(C) receptors increases light responsiveness of retinal ganglion cells in a rat model of retinitis pigmentosa. Exp Eye Res 2012; 105:21-6. [PMID: 23085337].

53. Stone RA, Liu J, Sugimoto R, Capehart C, Zhu X, Pendrak K. GABA, experimental myopia, and ocular growth in chick. Invest Ophthalmol Vis Sci 2003; 44:3933-46. [PMID: 12939312].

54. Schmid KL, Strasberg G, Rayner CL, Hartfield PJ. The effects and interactions of GABAergic and dopaminergic agents in the prevention of form deprivation myopia by brief periods of normal vision. Exp Eye Res 2013; 110:88-95. [PMID: 23474145].

55. Chebib M, Hinton T, Schmid KL, Brinkworth D, Qian H, Matos S, Kim HL, Abdel-Halim H, Kumar RJ, Johnston GA, Hanrahan JR. Novel, potent, and selective GABAC antagonists inhibit myopia development and facilitate learning and memory. J Pharmacol Exp Ther 2009; 328:448-57. [PMID: 18984654].

56. Cheng ZY, Wang XP, Schmid KL, Han XG. Inhibition of form-deprivation myopia by a GABAA0r receptor antagonist, (1,2,5,6-tetrahydropyridin-4-yl) methylphosphonic acid (TPMPA), in guinea pigs. Graefes Arch Clin Exp Ophthalmol 2014; 252:1939-46. [PMID: 25120102].

57. Leung CKS, Yeung CK, Chiang SWY, Chan KP, Pang CP, Lam DSC. GABAA and GABAC (GABAA0r) receptors affect ocular growth and form-deprivation myopia. Cutan Ocul Toxicol 2005; 24:187-96.

58. McFadden SA, Gambrill R, Leotta AJ, Bowrey HE, Zeng G. Inhibition of GABAc Slows Ocular Growth and Myopia in the Mammalian Eye. ARVO Annual Meeting; 2011 May 4-9; Fort Lauderdale, FL.

59. Barathi VA, Chaurasia SS, Poidinger M, Koh SK, Tian D, Ho C, Iuvone PM, Beuerman RW, Zhou L. Involvement of GABA transporters in atropine-treated myopic retina as revealed by iTRAQ quantitative proteomics. J Proteome Res 2014; 13:4647-58. [PMID: 25211393].

60. McBrien NA, Jobling AI. Gentle A. Biomechanics of the sclera in myopia: extracellular and cellular factors. Optom Vis Sci 2009; 86:E23-30. [PMID: 19104466].

61. Clapham DE. Calcium signaling. Cell 2007; 131:1047-58. [PMID: 18083096].

62. Smith-Thomas L, Haycock JW, Metcalfe R, Boulton M, Ellis S, Rennie IG, Richardson PS, Palmer I, Parsons MA, Mac Neil S. Involvement of calcium in retinal pigment epithelial cell proliferation and pigmentation. Curr Eye Res 1998; 17:813-22. [PMID: 9723997].

63. Gahramani M, Yousefi R, Khoshanian K, Alavianmehr MM. The impact of calcium ion on structure and aggregation propensity of peroxynitrite-modified lens crystallins: New insights into the pathogenesis of cataract disorders. Colloids Surf B Biointerfaces 2015; 125:170-80. [PMID: 25486325].

64. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 2003; 4:552-65. [PMID: 12838338].

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