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$GABA_{A\alpha1}$ and $GABA_{A\rho1}$ subunits are expressed in cultured human RPE cells and $GABA_A$ receptor agents modify the intracellular calcium concentration

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Purpose: *Gamma-aminobutyric acid*$_A$ ($GABA_A$) receptors ($GABA_A$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 ($[Ca^{2+}]i$). $GABA_A$R β-subunit protein has been isolated in the cultured human and rat RPE, and $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ mRNAs and proteins are present in the chick RPE. The purpose of this study was to investigate the expression of $GABA_{A\alpha1}$ and $GABA_{A\rho1}$, two important subunits in forming functional $GABA_A$Rs, in the cultured human RPE, and further to explore whether altering receptor activation modifies $[Ca^{2+}]i$.

Methods: Human RPE cells were separately cultured from five donor eye cups. Real-time PCR, western blots, and immunofluorescence were used to test for $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ mRNAs and proteins. The effects of the $GABA_A$R agonist muscimol, antagonist picrotoxin, or the specific $GABA_{A\rho}$ antagonist 1,2,5,6-tetrahydropyridin-4-yl) methylphosphinic acid (TPMPA) on $[Ca^{2+}]i$ in cultured human RPE were demonstrated using Fluo3-AM.

Results: Both $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ mRNAs and proteins were identified in cultured human RPE cells; antibody staining was mainly localized to the cell membrane and was also present in the cytoplasm but not in the nucleus. Muscimol (100 µM) caused a transient increase of the $[Ca^{2+}]i$ in RPE cells regardless of whether Ca^{2+} was added to the buffer. Muscimol-induced increases in the $[Ca^{2+}]i$ were inhibited by pretreatment with picrotoxin (300 µM) or TPMPA (500 µM).

Conclusions: $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ are expressed in cultured human RPE cells, and $GABA_A$ agents can modify $[Ca^{2+}]i$.

Gamma-aminobutyric acid$_A$ ($GABA_A$) receptors ($GABA_A$Rs), which form a subclass of receptors of the inhibitory neurotransmitter GABA, are ionotropic receptors involving chloride channels that mediate fast synaptic inhibition when activated by GABA [1]. $GABA_A$Rs include 19 subunits (alpha 1–6, beta 1–3, gamma 1–3, delta, epsilon, theta, pi, and rho 1–3) [2]. Most native $GABA_A$Rs are thought to consist of two alpha, two beta, and one gamma or delta subunits, and some $GABA_A$Rs can be formed from homo- or heteropentamers composed of rho subunits [3]. The $GABA_A$Rs being formed from rho subunits are also called $GABA_{AOr}$ receptors (previously termed $GABA_C$ receptors) [2,3].

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$GABA_A$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, $GABA_A$R B-chain protein has been detected in human corneal stem cells [13] and the $GABA_A$R β-subunit ($GABA_{A\beta}$) protein in the cultured human RPE [14]. In animal models, the $GABA_A$R beta 3 subunit protein has been identified in cultured mouse lens epithelial cells [15], $GABA_{A\beta}$ protein isolated from the cultured rat RPE [14], and $GABA_A$R alpha $_1$ ($GABA_{A\alpha1}$) and rho $_1$ subunit ($GABA_{A\rho1}$) mRNAs and proteins present in the chick RPE [16]; $GABA_{A\rho1}$ has also been visualized in the chick sclera [17].

$GABA_A$Rs have been reported to regulate intracellular calcium concentration ($[Ca^{2+}]i$) in a variety of cells. The

$GABA_AR$ agonist muscimol increases $[Ca^{2+}]i$ in rat astrocytes [18], as well as in embryonic and early postnatal neocortical cells [19], embryonic rat ventral spinal cord neurons [20], embryonic rat striatal neurons [21], and rat cerebellar Purkinje neurons [22]. It also alters $[Ca^{2+}]i$ in rat pituitary lactotrophs [23], immortalized gonadotropin -releasing hormone neurons [24], and alphaT3–1 gonadotropes [25]. Within ocular tissues, muscimol increases $[Ca^{2+}]i$ in postnatal mouse retinal ganglion cells [26] and mouse lens epithelial cells [15]; these increases have been prevented by $GABA_AR$ antagonists bicuculline and picrotoxin.

The RPE is a single layer of predominantly hexagonal, pigmented cells that interact apically with the interphotoreceptor matrix and the photoreceptor outer segments and basally with the Bruch's membrane of the vascular choriocapillaris (reviewed in [27]). Ca^{2+} signals play essential roles in the function of the RPE [28,29], and a normal $[Ca^{2+}]i$ appears to be essential if the RPE is to conduct its normal retinal maintenance functions [30]. Abnormal $[Ca^{2+}]i$ levels in the RPE have been reported to be associated with high lipofuscin formation [31], retinal dystrophy [32], and cell death [33]. The $[Ca^{2+}]i$ in RPE can be modified by various neural transmitter receptors, for example, acetylcholine muscarinic receptors [34], $alpha_7$ nicotinic acetylcholine receptors [35], adrenergic receptors [36,37], and $GABA_B$ receptors [38]. Whether stimulation of $GABA_ARs$ modifies the $[Ca^{2+}]i$ in RPE is unknown. The purpose of this study was to investigate the expression of $GABA_{A\alpha1}$ and $GABA_{A\rho1}$—two important subunits in forming functional $GABA_ARs$ [3]—and the effects of the $GABA_AR$ agonist muscimol, antagonist picrotoxin, and specific $GABA_{A\rho}$ antagonist TPMPA [39] on the $[Ca^{2+}]i$ in the cultured human RPE.

METHODS

Human RPE cell culture: 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 [43], or choroidal melanocytes [45], 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 $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ were designed and ordered from Shanghai Biosune Biotechnology Company (Shanghai, China). The primer sequences of human $GABA_{A\alpha1}$ were F: 5'- ACT TTT CAG CTG CTC CAG CCC G-3', R 5'- CTC CCA ATC CTG GTC TCA GGC GA-3'. The sequences of human GABAAρ1 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 $GABA_{A\alpha1}$ antibody (1:100, Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal goat anti-human $GABA_{A\rho1}$ antibody (1:100, Santa Cruz, CA), or monoclonal mouse anti-human β-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™ 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 65 antibody (1:300), monoclonal mouse anti-human S100 antibody (1:200), polyclonal goat anti-human $GABA_{A\alpha1}$ antibody (1:50), and polyclonal goat anti-human $GABA_{A\rho1}$ 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 [Ca^{2+}]i: [Ca^{2+}]i was measured as previously described [48]; 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 $GABA_AR$ agonist muscimol (100 μM; Sigma, MO) was added to the assay at set time points. To determine the impact of the $GABA_AR$ antagonist, cells were preincubated with either the $GABA_AR$ antagonist picrotoxin (300 μM; Sigma) or the $GABA_{A\rho}$ antagonist TPMPA (500 μM; Sigma) for 10 min before muscimol (100 μM) was added. To determine the effects of muscimol on [Ca^{2+}]i in buffer without Ca^{2+}, cells were rinsed and kept in PSS without 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 Ca^{2+} or without 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].

$GABA_{A\alpha1}$ and $GABA_{A\rho1}$ mRNA expression in cultured human RPE: Real-time PCR showed that $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ 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 ($GABA_{A\alpha1}$), 263 bp ($GABA_{A\rho1}$), and 302 bp (β-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 $GABA_{A\alpha1}$ and $GABA_{A\rho1}$.

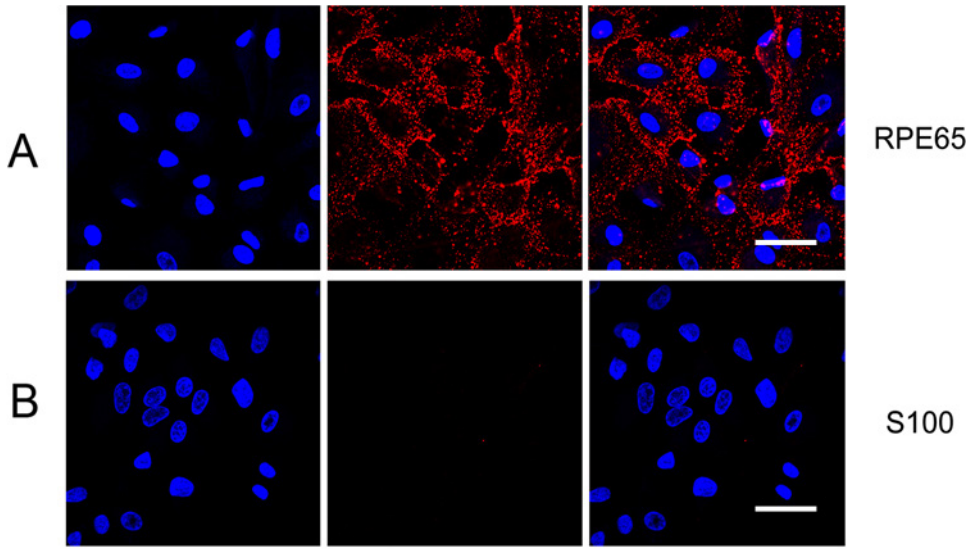

Figure 1. Phenotype identification of the cultured human RPE cells using immunofluorescence (representative image; n = 5). **A**: All of the cultured cells were positively stained with the RPE65 antibody. **B**: All of the cultured cells were negatively stained with the S100 antibody. Nuclei were stained by 4',6-diamidino-2-phenylindole (DAPI). Scale bar = 50 µm.

$GABA_{A\alpha1}$ and $GABA_{A\rho1}$ 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-$GABA_{A\alpha1}$, anti-$GABA_{A\rho1}$, and anti-β-Actin antibody, respectively, in cultured human RPE and retinal samples (positive control; Figure 3A).

Immunofluorescence revealed immunoreactivity to antibodies for $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ 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).

$GABA_AR$ agents modify the $[Ca^{2+}]i$ in cultured human RPE: There were no significant differences in baseline fluorescent intensity across treatment groups in the buffer either with Ca^{2+} (one-way ANOVA, p>0.05, n=5), or without Ca^{2+} (one-way ANOVA, p>0.05, n=5). The $GABA_AR$ agonist muscimol (100 µM) induced a rapid and significant $[Ca^{2+}]i$ increase in the cultured human RPE cells in the buffer either with Ca^{2+} (paired t-test, p<0.05, n=5; Figure 4A) or without Ca^{2+} (paired t-test, p<0.05, n=5; Figure 4D). The $[Ca^{2+}]i$ 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 $GABA_AR$ antagonist picrotoxin (300 µM; Figure 4B,E) or the $GABA_{A\rho}$ antagonist TPMPA (500 µM; Figure 4C,F), the muscimol (100 µM) induced $[Ca^{2+}]i$ increase was completely blocked (both when the buffer contained Ca^{2+}, Figure 4B,C, and when it did not, Figure 4E,F). The addition of the control agent, PBS, did not alter the $[Ca^{2+}]i$ of the cultured human RPE cells (paired t-test, p>0.05, n=5).

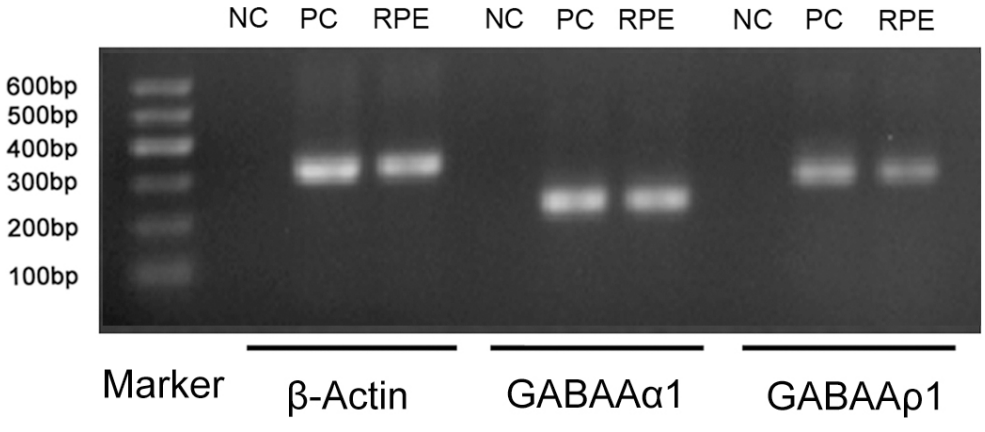

Figure 2. Sample ethidium bromide gel of real-time PCR products of gamma-aminobutyric $acid_{A\alpha1}$ ($GABA_{A\alpha1}$) and $GABA_{A\rho1}$ in cultured human RPE cells (representative image, n = 5). β–Actin (302 bp), $GABA_{A\alpha1}$ (211 bp); $GABA_{A\rho1}$ (263 bp). Abbreviations: NC, negative control; PC, human retina (positive control).

DISCUSSION

$GABA_{A\alpha1}$ and $GABA_{A\rho1}$ 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 $GABA_{A\rho}$ antagonist TPMPA. This suggests that both $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ occur in cultured human RPE cells and that in these cells, $GABA_A$R stimulation can modify $[Ca^{2+}]$i. This means that $GABA_{A\beta}$ [14], $GABA_{B1}$ and $GABA_{B2}$ [38], and now $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ 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

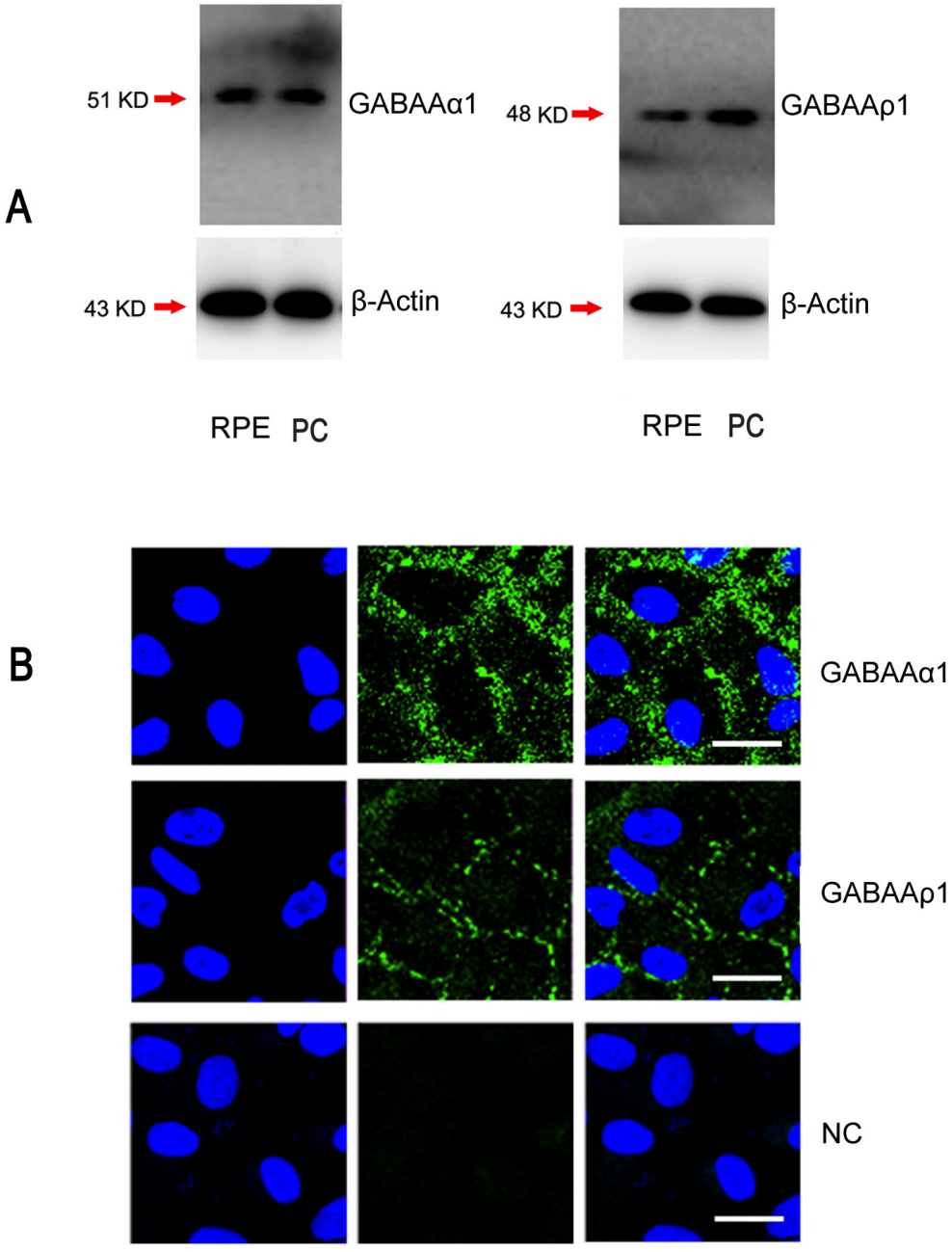

Figure 3. Gamma-aminobutyric $acid_{A\alpha1}$ ($GABA_{A\alpha1}$) and $GABA_{A\rho1}$ protein expression in cultured human RPE cells detected by western blots (A) and immunofluorescence (B) (representative image; n = 5). **A**: Specific bands presented at the approximate location of 51 kDa ($GABA_{A\alpha1}$), 48 kDa ($GABA_{A\rho1}$), and 43 kDa (β-Actin) in lysates of RPE and the retina (positive control, PC). **B**: Immunofluorescence staining of $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ in cultured human RPE. Nuclei were stained by 4',6-diamidino-2-phenylindole (DAPI; blue; bar = 20 µm). NC, negative control.

antagonist picrotoxin has been shown to attenuate the muscimol-induced [Ca^{2+}]i increase in postnatal mouse retinal ganglion cells [26]. In this study, we observed that the $GABA_AR$ agonist muscimol increase the [Ca^{2+}]i in cultured human RPE cells and that the antagonist picrotoxin blocked this rise. This suggests that activation of the $GABA_AR$ modifies the [Ca^{2+}]i in RPE cells as it does in retinal ganglion cells [26].

In the retina, the $GABA_{A\rho}$ 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^{2+}]i increase in cultured human RPE cells; suggesting that the $GABA_{A\rho}$ receptor functions are not unique to the retina.

$GABA_ARs$ 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 $GABA_AR$ agonist muscimol induced myopia development in chicks [53] and prevented the myopia-reducing effects of normal vision [54], whereas the $GABA_{A\rho}$ 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 $GABA_ARs$ are expressed in retina [4], and the $GABA_AR$ agonist muscimol and the $GABA_{A\rho}$ 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 $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ are expressed in the human RPE, and that muscimol and TPMPA modulate the [Ca^{2+}]i. 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 $GABA_ARs$ 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^{2+}]i of RPE cells. Ca^{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

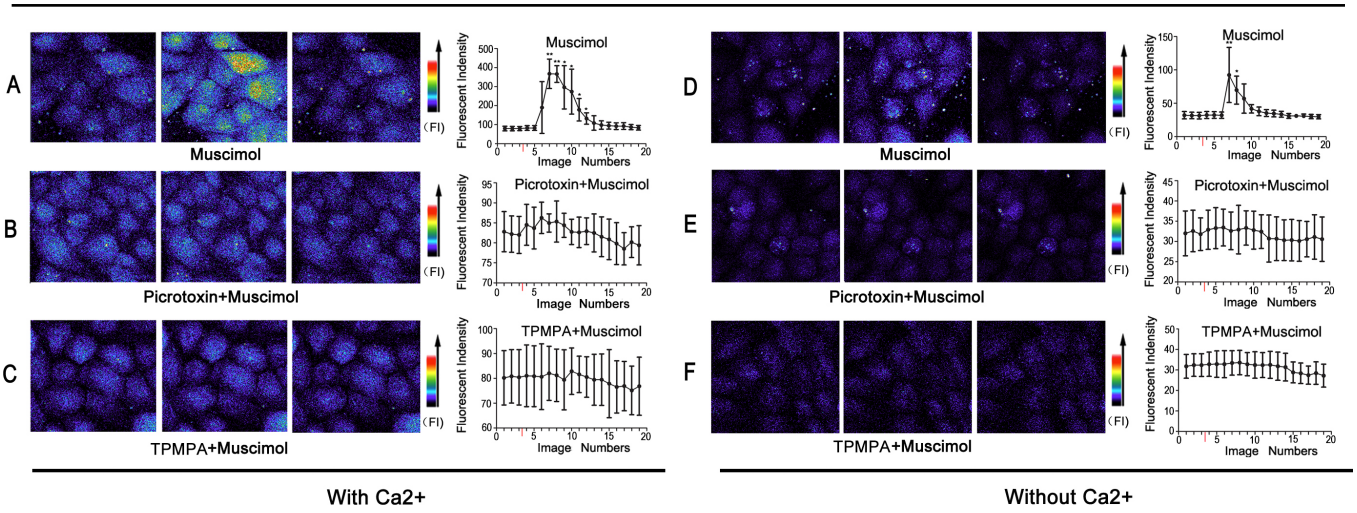

Figure 4. Evidence that the gamma-aminobutyric $acid_A$ receptor ($GABA_AR$) agonist muscimol, the antagonist picrotoxin and the $GABA_{A\rho}$ antagonist 1,2,5,6-tetrahydropyridin-4-yl) methylphosphinic acid (TPMPA) can modify the intracellular calcium concentration ([Ca^{2+}]i) in the cultured human RPE. **A**: Muscimol (100 µM) increased the [Ca^{2+}]i in cultured human RPE cells in buffer with Ca^{2+} (paired t-test, $p<0.05$). **B**: Pretreatment with picrotoxin (300 µM) completely inhibited the muscimol (100 µM) induced increase of [Ca^{2+}]i in buffer with Ca^{2+}. **C**: Pretreatment with TPMPA (500 µM) completely blocked the muscimol (100 µM) induced increase of [Ca^{2+}]i in buffer with Ca^{2+}. **D**: Muscimol (100 µM) increased the [Ca^{2+}]i in cultured human RPE cells in buffer without Ca^{2+} (paired t-test, $p<0.05$). **E**: Pretreatment with picrotoxin (300 µM) completely inhibited the muscimol (100 µM) induced increase of [Ca^{2+}]i in buffer without Ca^{2+}. **F**: Pretreatment with TPMPA (500 µM) completely blocked the muscimol (100 µM) induced increase of [Ca^{2+}]i in buffer without Ca^{2+}. Left, representative images (1, 8, and 15 images were acquired, respectively). Right, fluorescent intensity in the images acquired before and after muscimol application. The red bar represents the time when muscimol was added. FI represents the fluorescence intensity color scale, with the direction of the arrow indicating higher intensity. *indicates a $p<0.05$, and ** indicates a $p<0.01$, compared to the baseline, paired t-test, n=5 for each group.

[62]. 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, $GABA_{A\alpha1}$ and $GABA_{A\rho1}$ mRNAs and proteins were expressed in cultured human RPE cells, and the $GABA_AR$ agonist muscimol, antagonist picrotoxin, and $GABA_{A\rho}$ antagonist TPMPA were shown to modify [Ca^{2+}]i.

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