Vitamin A aldehyde-taurine adducts function in photoreceptor cells

Hye Jin Kim a, Jin Zhao a, Janet R. Sparrow a, b, *

a Departments of Ophthalmology and Columbia University Medical Center, New York, NY, 10032, USA
b Departments of Pathology and Cell Biology, Columbia University Medical Center, New York, NY, 10032, USA

ARTICLE INFO

Keywords:
Taurine
Vitamin A
Retinaldehyde
Bisretinoids
Retina

ABSTRACT

To facilitate the movement of retinoids through the visual cycle and to limit nonspecific chemical reaction, multiple mechanisms are utilized to handle these molecules when not contained within the binding pocket of opsin. Vitamin A aldehyde is sequestered by reversible Schiff base formation with phosphatidylethanolamine (PE) and subsequently undergoes NADPH-dependent reduction. Otherwise inefficient handling of retinaldehyde can lead to the formation of fluorescent di-retinal compounds within the outer segments of photoreceptor cells. These bisretinoid fluorophores initiate photooxidative processes having adverse consequences for retina. Various carrier proteins confer water solubility and maintain the 11-cis-retinoid configuration. Mechanisms for sequestration of retinoid include the formation of a reversible Schiff base between retinaldehyde and taurine (A1-taurine, A1T), the most abundant amino acid in photoreceptor cells. Here we have undertaken to examine the effects of taurine depletion using the transport inhibitors guanidinoethyl sulfonate (GES) and β-alanine. Oral treatment of BALB/cJ mice with β-alanine reduced ocular A1T and the mice exhibited significantly lower scotopic and photopic a-wave amplitudes. As a secondary effect of retinal degeneration, A1T was not detected and taurine was significantly reduced in mice carrying a P23H opsin mutation. Treatment of agouti and albino mice with GES and β-alanine. Oral treatment of BALB/cJ mice with β-alanine reduced ocular A1T and the mice exhibited significantly lower scotopic and photopic a-wave amplitudes. As a secondary effect of retinal degeneration, A1T was not detected and taurine was significantly reduced in mice carrying a P23H opsin mutation. Treatment of agouti and albino mice with GES and β-alanine was more pronounced in β-alanine treated mice. Treatment of agouti and albino Abca4−/− mice with β-alanine and GES was associated with reduced bisretinoid measured chromatographically. Consistent with a reduction in carbonyl scavenging activity by taurine, methylglyoxal-adducts were also increased in the presence of β-alanine. Taken together these findings support the postulate that A1T serves as a reservoir of vitamin A aldehyde, with diminished A1T explaining reduced photoreceptor light-sensitivity, accentuated ONL thinning in albino Abca4−/− mice and attenuated bisretinoid formation.

1. Introduction

Taurine (2-aminoethanesulfonic acid) is an endogenous sulfur-containing β-amino acid that is not incorporated into protein but is the most abundant constituent of the amino acid pool of photoreceptor cells [1]. Although taurine can be synthesized endogenously from cysteine, it is primarily acquired through the diet [1,2]. The structure of taurine does not include the carboxyl group typical of amino acids; instead, it bears a sulfonate group. Retinal pigment epithelial (RPE) cells take up considerable amounts of taurine; only the uptake of proline is higher [3]. Taurine enters RPE cells by means of a high-affinity Na+ and Cl− dependent taurine transporter (TauT; SLC6a6) [4–6]. High concentrations of free taurine accumulate in neural retina in association with photoreceptor cells [1]. Structural analogs of taurine, including the synthetic compound guanidinoethyl sulfonate (GES) [7] and natural occurring β-amino acids such as β-alanine [8] are competitive inhibitors of the taurine transporter [6,9]. Both glutathione (GSH) and taurine rely on cysteine for synthesis. It is perhaps for this reason that taurine supplementation may increase reduced GSH levels [10]. Aging is reported to be associated with decreased taurine in plasma and tissues [11].

Taurine deficiency negatively impacts the retina. Recently, there have been two reports of unrelated families with affected siblings carrying homozygous disease-causing missense variants (p.G399V; p.A78E) in the solute carrier (SLC) 6a6 gene [5,12] that encodes TauT [2]. These hypomorphic alleles conferred profound reductions in taurine transport [12], meager plasma taurine levels, cardiomyopathy and early-onset

Abbreviations: ERG, electroretinography; HPLC, high performance liquid chromatography; MG, methylglyoxal; ONL, outer nuclear layer; qAF, quantitative fundus autofluorescence; RPE, retinal pigment epithelial; UPLC, ultra performance liquid chromatography.

* Corresponding author. Departments of Ophthalmology and Columbia University Medical Center, New York, NY, 10032, USA.

E-mail address: jrs88@columbia.edu (J.R. Sparrow).

https://doi.org/10.1016/j.redox.2022.102386
Received 18 May 2022; Received in revised form 17 June 2022; Accepted 24 June 2022
Available online 3 July 2022
2213-2317/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
retinal degeneration. The latter was evidenced by thinning of photoreceptor-attributable layers in spectral domain optical coherence tomography (SD-OCT) scans and severe reductions in electroretinograms [12]. Oral taurine supplementation (100 mg/kg/day, 2 years) enabled normal blood taurine levels, correction of the cardiomyopathy and non-progression of retinopathy in a 6-year-old [12]. Interest in taurine also originates from reports that the bilateral concentric visual field loss that is a side-effect of the anti-epileptic drug vigabatrin (GABA-transaminase inhibitor) is due to taurine depletion [2,13,14].

Taurine carries a primary amine that in retina reversibly binds to retinaldehyde thereby forming a small molecule, taurine-retinaldehyde Schiff base (AIT) [16,17]. A1T is an amphiphilic molecule carrying a negatively charged sulfonated group together with a hydrophobic retinoid moiety and may serve to chaperone retinaldehyde, a molecule that is otherwise poorly soluble in aqueous media. We recently showed that AIT is present at higher levels in neural retina as opposed to RPE and is more abundant under conditions in which 11-cis-retinaldehyde is more abundant [16,17]. These conditions included mice carrying the Rpe65-Leu450 versus Rpe65-450Met variant, the presence versus absence of ocular pigmentation, dark-adaptation versus light-adaptation. A1T was also substantially decreased in mouse models in which the synthesis of 11-cis-Rpe65-Leu450, was bred in the laboratory and housed under 12-h on-off cyclic lighting with in-cage illumination of 30–80 lux. To achieve dark-adapted conditions, mice were placed in darkness for 18–20 h. Genotyping confirmed that the mutant mice do not carry the rd1, 2, 3, 6, 7, 8 and 10 mutations [26]. Mouse lines studied are provided in Table 1. The research was approved by the Institutional Animal Care and Use Committee (IACUC) and was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

2.1. Mouse models

BALB/cJ mice and P23H knock-in mice (Rhoda2+/-P23H) homozygous mice [25] were purchased from the Jackson Laboratory (Bar Harbor, ME). The albino Rhoda2+/- mice were custom generated at The Jackson Laboratory by repeated crossing homozygous black P23H mice (JAX stock# 017628) to albino B6 mice (JAX stock# 000058) for over 12 generations and the genetic background was confirmed to be over 99% of that of albino B6. Both albino homozgyous mice and their littermate controls were maintained in house. Albino Abca4 null mutant mice (Abca4-/-); albino Abca4+/+ mice; and agouti Abca4 null mutant mice (Abca4-/-) (The Jackson Laboratory) all of which were homozgyous for Rpe65-Leu450, were bred in the laboratory and housed under 12-h on-off cyclic lighting with in-cage illumination of 30–80 lux. To achieve dark-adapted conditions, mice were placed in darkness for 18–20 h. Genotyping confirmed that the mutant mice do not carry the rd1, 2, 3, 6, 7, 8 and 10 mutations [26]. Mouse lines studied are provided in Table 1. The research was approved by the Institutional Animal Care and Use Committee (IACUC) and was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

2.2. Taurine, β-alanine and GES treatment

Mice received oral taurine (Millipore-Sigma, St. Louis, MO; pharmagrade) delivered as 2.5% (w/v) in drinking water for 2 and 6 months. Guanidinoethyl sulfonate (GES) was synthesized by Toronto Research Chemicals (Toronto, Ontario; pharmagrade) and delivered in drinking water (1%) for 2 months. β-alanine (NutriVitaShop, Lake Forest, CA; pharmagrade) was delivered to mice in drinking water (2%) for 1–2 months.

2.3. In vitro cellular experiments

Human adult RPE (ARPE-19, American Type Culture Collection, Manassas, VA) were treated with GES and β-alanine where indicated. To test for direct effects of taurine on cultured RPE, ARPE-19 cells deficient in endogenous lipofuscin [27] were grown to confluence as described [28]. The cells accumulated A2E with and without β-alanine for 14 days. After culturing for an additional 3 days in media without A2E, the cells were incubated with taurine (1 mM) for 24 h and without further incubation the cells were exposed to 430-nm light (30 min). After 18 h, the MTT (4, 5-di-methylthiazol-2-y)-2, 5-diphenyltetrazolium bromide) assay (Roche Diagnostics, Indianapolis, IN) was performed to evaluate cell viability.

2.4. Synthesis

A2E, A2-GPE (A2-glycerophosphoethanolamine), A2-DHP-PE (A2-dihydropropyridine-phosphatidylethanolamine), αtRALdi-PE (all-trans-retinal dimer-phosphatidylethanolamine) and αtRALdi were synthesized as previously described with HPLC purification [23,27,29,30]. Retinoids (9-cis-retinal, 13-cis-retinal and all-trans-retinal) were purchased from Sigma-Aldrich; 11-cis-retinal was a gift from the National Eye Institute provided through the laboratory of Dr. Rosalie K. Crouch. A1-taurine isomers were synthesized as published [21]. A1-taurine isomers were purified in their protonated forms by HPLC with a reverse-phase Atlantis Prep dC18 column (10 × 250 mm, 10 μm; Waters) and gradient of acetonitrile and water with 0.1% TFA [17].

2.5. Quantitative UPLC analysis of retinoids

Frozen mouse eyecups (1 eye/sample) were homogenized and derivatized with O-ethylhydroxylamine (100 mM) (method 1) or with O-ethylhydroxylamine (100 mM) and acetonitrile (method 2) on ice under dim red light. Retinoids were extracted with hexane and resuspended in acetonitrile for UPLC analysis [32] using a Waters Acquity UPLC-PDA system (Milford, MA) with a CSH C18 column as described [33]. Molar quantities per eye were calculated based on standard solutions with concentrations determined spectrophotometrically. Peak areas were calculated using Waters Empower Software and results were analyzed in Excel (Microsoft, Redmond, WA).
2.6. Quantitation of bisretinoids

Mouse eyecups were homogenized, extracted with either A or B (A: homogenized in Dulbecco’s phosphate-buffered saline (DPBS) and extracted in chloroform/methanol, 1:1; B: homogenized and extracted in chloroform/methanol, 1:1) and the solvent was evaporated as previously described [34]. The extract was re-dissolved in chloroform/methanol by HPLC and in ethanol by UPLC for bisretinoid measurement (Waters, Milford, MA) [35]. One to eight eyes were combined for bisretinoid measurement, each measurement was expressed as picomoles/eye, and from multiple measurements, mean values were determined.

2.7. HPLC quantitation of taurine

RPE cells and whole murine eyes or isolated neural retina and isolated RPE/choroid were homogenized in DPBS and extracted in chloroform and methanol (1:1) to exclude the non-polar fraction. The DPBS fraction was derivatized with NBD-F solution (1 mmol/L) as described previously [36]. An Alliance system (Waters, Milford, MA) equipped with a 2475 Multi λ fluorescence detector (Ex: 470 nm, Em: 530 nm) was used for HPLC analysis as previously described [17]. Molar quantity of taurine was determined using standard curves.

2.8. UPLC quantitation of A1T isomers

Whole mouse eyes (3–4 eyes/sample) were homogenized in acidified MeOH (0.1% TFA) and extracted in chloroform/DPBS (2: 1.3) (on ice and under dim red light). Acidified methanol was used to protonate the primary amine of taurine thus rendering it incapable of nucleophilic attack on the aldehyde moiety of retinaldehyde. The addition of PBS in the biphasic system with chloroform prevented unwanted formation of Schiff base during extraction [17]. The sample was then centrifuged, filtered, and extracted twice by addition of chloroform. The combined chloroform fraction was dried and then redissolved in MeOH and measured by a Waters Acquity UPLC system (Milford, MA) with an XBridge BEH C18 column. A1-taurine isomers 11-cis, 13-cis, 9-cis, all-trans-retinal were separated with water and acetonitrile, both with 0.1% formic acid as published [17]. Molar quantities per eye were calculated by comparison with each of the synthesized A1T isomers. To calculate total A1T, the isomers were quantified separately and then summed [17]. A1-β-alanine was added as an internal standard to compensate for Schiff base hydrolysis during the extraction. In desiccated mouse eyes extracted in dehydrated methanol that favors reactions between endogenous taurine and β-alanine with free retinaldehyde we confirmed that most of the Schiff base was A1T (99%) and a small portion of Schiff base was A1-β-alanine (1%). This is consistent with previous findings [37].

2.9. Measurement of outer nuclear layer (ONL) thickness

Mouse eyes were marked and fixed as described [38]. Sagittal paraffin serial sections of mouse retina were prepared and stained with hematoxylin and eosin. The section most centrally positioned in the optic nerve head (ONH) was selected and imaged with the 40 x objective using Leica SCN 400 system (Leica Microsystems; Leica Application suite; Wetzlar, Germany). ONL thickness was then measured at 200 μm intervals superior and inferior to the edge of the ONH along the vertical meridian with image processing software Fiji after a custom ruler was placed with Photoshop CS5. ONL width in pixels was converted to microns (1 pixel: 1.26 μm). For groups of mice at the indicated ages, mean ONL thickness of 3 sections at each position along the vertical plotted as a function of eccentricity from the ONH [39].

2.10. Electoretinography (ERG)

ERG recordings were obtained with a Celeris rodent ERG system (Diagnosys, Lowell, MA). Scotopic and photopic responses were recorded under incremental stimuli. To measure recovery of visual sensitivity, the eyes were exposed to 1000 cd s/m² white light for 5 min to bleach rhodopsin (90%) and ERG responses to 1 cd s/m² white light were recorded before and after bleaching at regular intervals.

2.11. Quantitative fundus autofluorescence (qAF)

Short-wavelength (SW) fundus autofluorescence (488 nm excitation) images were acquired non-invasively from mice as described [40].

2.12. Detection of methylglyoxal-(MG) adducts by enzyme-linked immunosorbent assay (ELISA)

Dissected neural retina and RPE (8 eyes per sample) were placed in 1X lysis buffer (Cell Signaling, Danvers, MA) with 1% (v/v) protease inhibitors (protease inhibitor cocktail, Millipore-Sigma, St. Louis, MO) and were sonicated on ice. After centrifuging the lysates, the MG-H1 (methylglyoxal-hydroimidazolone) protein adducts were quantified by competitive indirect enzyme-linked immunosorbent assay (ELISA) using an HRP conjugated-secondary antibody (OxiSelect, Cell Biolabs, San Diego, CA). MG content was determined by comparison with an MG-BSA

---

Table 1

Mouse lines included in the study.

| Genotype          | Treatment in drinking water (w/v) | Age (months) | Light-condition | Measurement                  | Corresponding figure |
|-------------------|-----------------------------------|--------------|-----------------|------------------------------|----------------------|
| BALB/cj           | β-alanine 2%                      | 1–3          | cyclic light    | taurine in plasma            | Fig. 1B              |
|                   |                                   |              |                 | taurine in eyes              | Fig. 1C              |
|                   |                                   |              |                 | A1T                          | Fig. 1D              |
|                   |                                   | 1–3          | dark-adaptation | retinoids                    | Fig. 2A and B        |
|                   |                                   | 2–3          | light-adaptation| retinoids                    | Fig. 2C and D        |
|                   |                                   | 1–3          | cyclic light    | ERG                          | Fig. 3               |
|                   |                                   | 2–3          | 2 h post-bleach | retinoids                    | Fig. 4A and B        |
| Albino Rho<sup>+/+</sup>/Rho<sup>−/−</sup> | No treatment | 2            | cyclic light    | taurine in eyes              | Fig. 5A              |
|                   |                                   |              |                 | A1T                          | Fig. 5C              |
|                   |                                   | 4            | dark-adaptation | retinoids                    | Fig. 5D              |
|                   |                                   | 3            | cyclic light    | A1T                          | Fig. 5F              |
|                   |                                   |              |                 | bisretinoids                 | Fig. 6A-C            |
|                   |                                   |              |                 | bisretinoids                 | Fig. 6D and E        |
|                   |                                   |              |                 | bisretinoids                 | Fig. 6F and G        |
| albinou Abca<sup>4−/−</sup>/Abca<sup>4−/−</sup> | β-alanine 2% | 1–3          | cyclic light    | bisretinoids                 | Fig. 7               |
|                   |                                   |              |                 | ONL                          | Fig. 8               |

ERG, electoretinography; GES, guanidinoethanol sulfonate; ONL, outer nuclear layer.
standard curve, a four-parameter fit algorithm [41]. To ensure antibody specificity for MG-H1, an adduct of MG with an arginine residue and a reaction mixture of MG with taurine were screened.

2.13. Statistical analysis

Statistical analysis was carried out using GraphPad Prism, version 8 (GraphPad Software, Inc. La Jolla, CA) and P < 0.05 was considered significant.

3. Results

3.1. Depletion of taurine by GES- and β-alanine-treatment in ARPE-19 cells and in mice

The taurine transporter (TauT) a sodium- and chloride-dependent protein encoded by the SLC6A6 gene and expressed by RPE is competitively inhibited by analogs of taurine such as guanidinoethyl sulfonate (GES) and β-alanine [7,42]. Since expression of TauT by ARPE-19 cells has been demonstrated previously by immunocytochemical staining [43], we confirmed the inhibitory behaviors of β-alanine and GES by HPLC quantification of taurine in ARPE-19 cells that were grown in media (with 10% fetal bovine serum) containing 0.4 microM taurine and treated with 0.5 and 2 mM of β-alanine and GES. In these GES- and β-alanine-treated cells, taurine concentration, measured after derivatization of amine groups by NBD-F, was inhibited by 96% and 97%, respectively (P < 0.01, 1-way ANOVA and Tukey’s multiple comparison test) (Fig. 1A). We also quantified taurine in plasma and in isolated neural retina and RPE of mice. In cyclic reared albino BALB/cJ mice receiving β-alanine in drinking water from age 1–3 months, HPLC analysis revealed 19% lower levels of taurine in plasma (P < 0.05, unpaired two-tailed t-test) (Figs. 1B) and 14% decreased taurine in isolated neural retina (P < 0.001, two-way ANOVA and Bonferroni’s multiple comparison test) versus untreated-controls (Fig. 1C). Conversely, in isolated RPE there was no appreciable difference in taurine levels between control and treated mice (Fig. 1C). Since β-alanine and GES produced similar levels of inhibition, and β-alanine was more readily available, we continued the work using β-alanine.

3.2. A1T levels in β-alanine-treated WT mice

A1T was also measured in eyes of BALB/cJ mice raised under cyclic light and treated with β-alanine from 1 to 3 months of age. Ocular A1T was reduced by 35% (P < 0.01; unpaired two-tailed t-test) in the β-alanine-treated versus control mice (Fig. 1D).

3.3. Effect of β-alanine on retinoid levels

We have previously shown that levels of A1T are higher under conditions in which 11-cis-retinal is more abundant. For instance, A1T (sum of 9-cis, 11-cis, 13-cis and all-trans isomers) is significantly greater in dark-adapted albino mice (C57BL/6J) and BALB/cJ mouse relative to the same strains of cyclic light-adapted mice [17]. A1T is also higher in black C57BL/6J mice as compared to albino C57BL/6J mouse. In the current studies BALB/cJ mice were treated with β-alanine from 1 to 3 months of age and then dark-adapted. In the treated mice there were no differences in retinoid (atROL, atRE, atRAL, 11cisRAL, 11cisRE) levels as compared to untreated mice. In the β-alanine-treated mice, there was, however, a decrease in 11cisROL (28% decrease) (P < 0.05, two-way ANOVA, and Sidak’s multiple comparison test) (Fig. 2A and B). Similarly in cyclic-light reared BALB/cJ mice, there was no difference in retinoid levels in mice receiving oral β-alanine for 1 month compared with the untreated mice (Fig. 2C and D). We note that the estimated total retinoid concentration in dark vs light-adapted eyes is 500.9 ± 66.5 pmoles/eye and 700.6 ± 90.5 pmoles/eye, respectively (extraction method 1).

3.4. Electroretinographic recording in taurine-deficient mice

To assess retinal function in terms of both rod and cone activity, we also recorded scotopic and photopic electroretinographic responses in 3-month-old BALB/cJ mice treated with β-alanine for 2 months to reduce taurine uptake and we compared the data to untreated mice. The corneal-negative a-wave originates from photoreceptor cells while the b-wave reflects responses from bipolar cells [44]. As shown previously in taurine-deficient mice [45], β-alanine-treated mice exhibited significantly lower scotopic and photopic a-wave amplitudes (P < 0.05, 1-way ANOVA and Tukey’s multiple comparison test) (Fig. 3A), and significantly increased b-wave implicit times (P < 0.01, 1-way ANOVA and Tukey’s multiple comparison test) relative to control mice (Supplementary Fig. S1). B-wave amplitudes were unchanged (Fig. 3B). The changes in a-wave amplitude without corresponding changes in b-wave amplitudes likely indicates that the photoreceptor cell responses were adequate to elicit responses in bipolar cells; transmission of signal from photoreceptors to second order neurons is non-linear [46].

3.5. Effect of β-alanine on the recovery of retinoid levels 2, 6 h postbleach in dark-adapted BALB/cJ mice

Retinoids were analyzed in β-alanine-treated mice and controls when mice were dark-adapted overnight, subjected to photobleaching and allowed to recover for 2 and 6 h in the dark (Fig. 4). After 2 and 6 h of
3.6. A1T, retinoid and taurine levels in a mouse model of retinitis pigmentosa

A point mutation of proline to histidine at position 23 of opsin (P23H) is responsible for a dominant form of retinitis pigmentosa (RP) [47]. Partial misfolding of rhodopsin in P23H mutant mice results in abnormal 11-cis-retinal binding to the opsin protein [48]. In pigmented Rho<sup>P23H/+</sup> knock-in mice at age 63 days the ONL consists of 6–7 rows of photoreceptor cell nuclei as compared to 9–10 rows in wild-type mice [25]. We found that the concentration of taurine in the whole eyes (including neural retina) of albino albino Rho<sup>P23H/+</sup> mice was decreased by 31.7% when compared to Rho<sup>+/+</sup> control mice (Fig. 5A). Since numerous findings indicate that in retina taurine functioning primarily relates to photoreceptor cells [2,12,15,49–52], we tested for a secondary effect of reduced retinoid and photoreceptor cell degeneration on A1T levels. Accordingly, in albino Rho<sup>P23H/+</sup> knock-in mice at age 1 month we observed that 11-cis-retinal was reduced by 85% and atRAL was 69% lower (Fig. 5B). All-trans-retinyl ester, all-trans-retinal and 11-cis-retinal were also significantly reduced in the mutant mice (P < 0.0001, 2-way ANOVA and Sidak’s multiple comparison test) (Fig. 5B). A1T was measured and compared in albino cyclic light-housed mice. The latter taurine-retinaldehyde Schiff base [17], was not detected in ocular recovery, there were no significant differences in retinoid levels in BALB/cJ mice treated with β-alanine for 1 and 2 months (P > 0.05) (Fig. 4).

Fig. 2. UPLC quantitation of retinoids in β-alanine (2% in drinking water) treated and untreated (control) albino BALB/cJ mice (age 3 months). (A–D) Dark-adapted mice treated with β-alanine from age 1–3 months. (C, D) Cyclic light-adapted mice treated with β-alanine from age 2–3 months. On the left axis, all-trans-retinol (atROL), all-trans-retinyl palmitate (atRE), all-trans-retinal (atRAL), 11-cis-retinal (11cisRAL) are presented as picomoles per eye. On the right axis, 11-cis-retinol (11cisROL) and 11-cis-retinyl ester (11cisRE) are presented as peak area/eye. Extraction method 1. Individual values are based on one eye; n = 4–17, mean ± SD. P values were determined by two-way ANOVA and Sidak’s multiple comparison test; *P < 0.05.

Fig. 3. Electroretinographic analysis of albino BALB/cJ mice treated orally with β-alanine from age 1–3 months. (A) a-wave amplitudes. (B) b-wave amplitudes. Amplitudes are plotted as a function of light-stimulus intensity for scotopic and photopic ERG recordings. Mean ± SD, n = 6. P values were determined by one-way ANOVA and Tukey’s multiple comparison test. *P < 0.05; **P < 0.01.

Fig. 4. Photobleaching and recovery. UPLC quantitation of retinoids in BALB/cJ mice treated with β-alanine for 1 (A, B) and 2 (C, D) months to deplete taurine. Mice were exposed to bleaching light and then allowed to recover for 2 (A, B) and 6 (C, D) hours in the dark. On the left axis all-trans-retinol (atROL), all-trans-retinyl palmitate (atRE), all-trans-retinal (atRAL), 11-cis-retinal (11cisRAL) are presented as picomoles per eye. On the right axis 11-cis-retinol (11cisROL) and 11-cis-retinyl ester (11cisRE) are presented as peak area/eye. Extraction method 2. Individual values are based on one eye; n = 3–10, mean ± SD. P values were determined by two-way ANOVA and Sidak’s multiple comparison test.
samples (4 eyes per sample) of albino Rho<sup>P23H1±</sup> mice at age 8 weeks, whereas all-trans-A1T was detected in age-matched WT mice (P < 0.001, unpaired two-tailed t-test) (Fig. 5C).

3.7. Taurine, retinoid and A1T in Abca4<sup>+/−</sup> mice

The Abca4<sup>+/−</sup> mouse is a model of recessive Stargardt disease, a disorder of central retina that begins with deterioration of RPE and photoreceptor cells [53]. Impaired ABCA4 activity is associated with less efficient reduction of retinaldehyde to retinol [54] and in albino Abca4<sup>−/−</sup> mice photoreceptor cell degeneration is readily detectable at age 9 months [40]. Thus we also compared A1T levels in untreated albino Abca4<sup>−/−</sup> versus WT mice (age 3 months). No differences were observed when eyes were studied at age 3 months (Fig. 5F), an age at which photoreceptor cell loss has not yet occurred. Likewise, levels of retinoids (atROL, atRE, atRAL, 11-cisRAL) and taurine were not different observed when eyes were studied at age 3 months (Fig. 5F), an age at which photoreceptor cell loss has not yet occurred. Likewise, levels of retinoids (atROL, atRE, atRAL, 11-cisRAL) and taurine were not different

3.8. Decreased cellular taurine by β-alanine-treatment results in reduced bisretinoid levels in agouti and albino Abca4<sup>−/−</sup> mice

Depletion of ocular taurine by β-alanine resulted in reduced bisretinoid levels in albino and agouti Abca4<sup>−/−</sup> mice (Fig. 6A-G). In albino Abca4<sup>−/−</sup> mice housed in cyclic light and treated with β-alanine from 1 to 3 months of age, levels of A2E, A2-GPE, A2-DHP-PE, and a1RALDi measured in whole eyes were reduced by 39.3%, 37.6%, 48.1% and 77.8% (P < 0.01, 2-way ANOVA and Sidak’s multiple comparison test), respectively relative to untreated albino Abca4<sup>−/−</sup> mice (Fig. 6B). The sum of all bisretinoids in the β-alanine-treated mice was 44.4% lower (P < 0.01, unpaired two-tailed t-test) (Fig. 6C). Treatment of agouti Abca4<sup>−/−</sup> mice with oral β-alanine or β-alanine for 2 months before euthanasia at 3 months also reduced total bisretinoids by 55.2% (P < 0.05, unpaired two-tailed t-test) (Fig. 6D). The individual bisretinoid A2E (A2E and isomers) was significantly reduced by 60.7% (P < 0.05, 2-way ANOVA and Sidak’s multiple comparison test) (Fig. 6D) after GES treatment and was reduced by 44.6% after β-alanine treatment (P < 0.05, 2-way ANOVA and Sidak’s multiple comparison test) (Fig. 6F).

3.9. Taurine supplementation in mice does not alter bisretinoid levels

To test for an effect of taurine supplementation, we measured bisretinoids in 3 month-old albino Abca4<sup>−/−</sup> mice after taurine was delivered in drinking water for 2 months. No statistically significant change was observed between control and supplemented mice (Fig. 7A).

Since bisretinoids are the source of fundus autofluorescence (fundus AF) elicited with 488 nm excitation, we also measured bisretinoids in albino Abca4<sup>−/−</sup> mice by non-invasive quantitative fundus autofluorescence (qAF) [55]. Oral delivery of taurine from age 1–3 months had no effect on qAF levels (Fig. 7B).

3.10. Outer nuclear layer thickness in taurine-deficient mice

Photoreceptor cell viability in albino Abca4<sup>−/−</sup> mice was assessed by measuring ONL thickness (Fig. 8). Importantly, the thinning of ONL that is indicative of reduced photoreceptor cell viability in albino Abca4<sup>−/−</sup> mice was more pronounced in mice treated with β-alanine from age 6–8 months mice (Fig. 8A). The ONL area, determined using the sum of ONL thicknesses in superior and inferior retina (2 mm), was decreased by 13% (P < 0.0001, unpaired two-tailed t-test) in mice receiving oral β-alanine compared with the control mice (Fig. 8C).

3.11. Quantitation of methylglyoxal-adduct proteins by ELISA

Photooxidation-induced and iron-mediated degradation of bisretinoid leads to the production of carboxyl-carrying molecular fragments such as methylglyoxal (MG) [21,56]. We have previously detected an adduct of MG with arginine residues (MG-derived hydroimidazolone: MG-H1), in posterior eyecups of agouti Rdh8<sup>−/−</sup>/Abca4<sup>−/−</sup> mice by indirect competitive ELISA [22] and corroborated this finding by
UPLC-MS detection of 2,4-dinitrophenylhydrazones (DNPH-derivatized) photocleavage products of the bisretinoid A2E [22]. Here too, we measured the production of MG-derived hydroimidazolone (MG-H1), an adduct of MG with arginine residues in isolated neural retina and RPE by indirect competitive ELISA. In albino Abca4−/− mice treated with β-alanine for 2 months MG-H1 was significantly increased by 23.2% in isolated neural retina relative to untreated controls (P < 0.01, 2-way ANOVA and Sidak’s multiple comparison test) (Fig. 9 A). As shown in Supplementary Fig. S2, taurine-MG adducts were not detected in a reaction mixture of MG with taurine. This control indicated that the ELISA assay is specific for MG-H1 adducts.

3.12. In vitro cellular photooxidation assay

To corroborate our findings in mice we also tested the effects of taurine in a known cell model using cell viability (MTT assay) as the outcome measure. Specifically, β-alanine (1 mM, 4 treatments over 2 weeks) was delivered to A2E-containing ARPE-19 after which the cells were exposed to 430-nm light to induce A2E photodegradation. Cell viability was reduced by 19% (P < 0.05, one-way ANOVA and Newman-Keuls multiple comparison test) (Fig. 9B) due to taurine deficiency. In a control experiment, delivery of β-alanine alone to ARPE-19 cells at a concentration of 0.1–1 mM for 2 weeks (4 treatments) did not cause the death of the cells as shown by MTT assay (Fig. 9 D).

We surmised that the absence of an effect of taurine supplementation in the mouse was attributable to taurine sufficiency in the mouse retinae. Indeed taurine-treatment (1 mM) was associated with a 24% increase in viability of ARPE-19 cells burdened by A2E accumulation and blue light exposure (P < 0.001, 1-way ANOVA and Tukey’s multiple comparison test) (Fig. 9C). Moreover, neither the addition of taurine in acellular assays nor depletion of the latter by treating ARPE-19 cells with the taurine transport inhibitors, GES, attenuated photooxidative loss of A2E, indicating that the effects of taurine are not mediated by inhibiting photooxidation (Supplementary Fig. S3). But more likely occur...
downstream at the stage of photodegradation (Supplementary Fig. S4).

4. Discussion

Although A1T was described several years ago, this taurine-retinaldehyde Schiff base has been largely ignored. We approached this study with the knowledge that numerous findings indicate that in retina taurine function primarily relates to photoreceptor cells. In this work we have demonstrated that depletion of taurine by selective inhibition of the taurine transporter TauT leads to reduced taurine in plasma and neural retina, reduced A1T in retina, reduced scotopic and photopic a-wave amplitudes and decreased bisretinoid lipofuscin levels in Abca4<sup>−/−</sup> mice. β-alanine-induced taurine deficiency was also associated with elevated MG adducts measured as MG-H1. In albino Rho<sup>P23H/−</sup> knock-in mice we found that at age 1 month 11-cis-retinal and all-trans-retinal were reduced by 85% and 69%, respectively. The concentration of ocular taurine in albino Rho<sup>P23H/−</sup> mice was decreased by 31.7% when compared to Rho<sup>/+/−</sup> control mice and A1T was not detectable. Previous studies in a dog model of RP also showed that taurine was reduced secondary to photoreceptor loss [57]. Likewise, a pronounced decline in taurine accompanies photoreceptor cell degeneration in the postnatal rd1 and C3H mouse [1,58] and photoreceptor cell degeneration induced by N-methyl-N-nitrosourea (MNU) [51]. On the other hand, destruction of inner retina has a minimal effect on the content of taurine in retina [1]. On the basis of our current findings and the previous findings of others, we surmise that with a loss of photoreceptor cells there is little need for taurine and A1T.

We also observed that in untreated Abca4<sup>−/−</sup> mice, there were no differences in ocular taurine, A1T nor retinoid levels as compared to wild-type mice at age 3 months. We note that at the latter age, loss of photoreceptor cells, as reflected in ONL thinning, is not observed [59]. However, ONL thinning that is detectable at 8 months of age in Abca4<sup>−/−</sup> mice was aggravated by β-alanine-depletion of taurine. This adverse effect on photoreceptor cells is consistent with previous findings. For instance in cats [60] raised on a diet that depletes taurine in serum and retina [49,50,61] and in mice carrying a homozygous deletion in the SLC6a6Tau gene encoding for TauT, the pronounced depletion of taurine leads to photoreceptor cell degeneration and absent ERG responses after 4 weeks of age [62]. Depletion of taurine (by 1% guanidinoethyl sulfonate) in albino rats is also associated with retinal degeneration that is exacerbated, particularly in central superior retina, by elevated environmental light [63]. In humans, a diet chronically deficient in taurine results in abnormal ERG recordings that can be reversed by intravenous delivery of taurine [64,65].
Bisretinoid formation in photoreceptor cells occurs due to unchecked retinaldehyde reactivity. Since taurine sequesters retinaldehyde via formation of the Schiff base A1T [16,17] one might have expected to find that taurine supplementation with the associated formation of A1T would reduce unwanted bisretinoid formation. Instead, we found that taurine-treatment had no effect on bisretinoid formation in young Abca4−/− mice. Conversely, depletion of ocular taurine by β-alanine resulted in reduced bisretinoid levels in albino and agouti Abca4−/− mice without a corresponding change in 11-cis-retinaldehyde levels. This differs from some other conditions under which reduced bisretinoid formation has been shown to follow from a reduction in serum retinol and 11-cis-retinal under light-adapted conditions [66,67]. Moreover, it has previously been discerned that reduced bisretinoid is associated with protection of photoreceptor cell viability [68]. Here however, β-alanine-associated reduction in taurine levels also attenuated bisretinoid levels while accentuating ONL thinning in formation has been shown to follow from a reduction in serum retinol-competitive indirect ELISA in isolated neural retina and RPE in albino mice. The finding that a decrease in ocular taurine resulted in lower bisretinoid findings suggest that taurine scavenges these dicarbonyls (Fig. S5) and that like glutathione [71] taurine may be bifunctional in photoreceptor cells. Scavenging activity such as this is also consistent with high taurine concentrations in retina [2].

As opposed to alleviating the adverse consequences of bisretinoid degradation we found in the current work that β-alanine-induced taurine-deficiency resulted in an increase in MG adducts measured as MG-H1. This finding suggests that taurine scavenges these dicarbonyls and that like glutathione [71] taurine may be bifunctional in photoreceptor cells. That said, it is also possible that the effect of diminished taurine levels on photoreceptor cell viability involves an additional mechanism. Specifically taurine may serve to scavenge dicarbonyls associated with an increase in MG adducts measured as MG-H1.

Toxidrinal degradation we found in the current work that β-alanine-induced taurine-deficiency resulted in an increase in MG adducts measured as MG-H1. This finding suggests that taurine scavenges these dicarbonyls and that like glutathione [71] taurine may be bifunctional in photoreceptor cells. That said, it is also possible that the effect of diminished taurine levels on photoreceptor cell viability involves an additional mechanism. Specifically taurine may serve to scavenge dicarbonyls released by bisretinoid photodegradation.

5. Conclusion

While taurine may be multi-functional, the reversible Schiff base A1T likely has visual cycle retinoids as its focus. A1T is an amphiphilic small molecule carrying a negatively charged sulfonated group together with a hydrophobic retinoid moiety. Because of its hydrophilic moiety, A1T has the potential to reside in a milieu distinct from NRPE. Engagement of A1T in a cone-driven visual cycle could account for the low levels of 11-cis-A1T in mouse since in this rodent only 5% of photoreceptor cells are cones. If A1T constitutes a pool of mobilizable 11-cis-retinal as we have suggested [17], a β-alanine-induced deficit in taurine could reduce the availability of retinaldehyde, an effect that would manifest as diminished ERG responses. Reduced visual chromophores may account for ONL thinning and would attenuate bisretinoid formation in photoreceptor cells. That said, it is also possible that the effect of diminished taurine levels on photoreceptor cell viability involves an additional mechanism. Specifically taurine may serve to scavenge dicarbonyls released by bisretinoid photodegradation.

Data availability

All relevant data are provided in the paper and supporting information file.
Funding
This work was supported by grants from the National Institute [EY012951; and P30EY019007] and Research to Prevent Blindness to the Department of Ophthalmology, Columbia University Medical Center.

Declaration of competing interest
Authors (Hyé Jin Kim, Jin Zhao, and Janet R. Sparrow) do not have conflicts of interest.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/redox.2022.102386.

References
[1] H. Rippis, W. Shen, Review: taurine: a “very essential” amino acid, Mol. Vis. 18 (2012) 2673–2688.
[2] N. Foger, et al., Taurine: the comeback of a neuroactive compound in the prevention of retinal degenerations, Prog. Retin. Eye Res. 41 (2014) 44–63.
[3] J.R. Chao, et al., Human retinal pigment epithelial cells prefer proline as a nutrient and transport metabolic intermediates to the retinal side, J. Biol. Chem. 292 (2017) 29653–29666.
[4] K.E. Smith, et al., Cloning and expression of a high affinity taurine transporter from rat brain, Mol. Pharmacol. 42 (1992) 563–569.
[5] M.N. Preising, et al., Biallelic mutation of human SLC6A6 encoding the taurine transporter TAUT is linked to early retinal degeneration, Faseb. J. 33 (2019) 11507–11527.
[6] S. Vinzakata, X. Qian, H. Egal, V. Sarthry, H.K. Sarkar, Molecular characterization and in situ localization of a mouse retinal taurine transporter, J. Neurochem. 69 (1997) 2236–2250.
[7] H. Pasantes-Morales, O. Quesada, A. Carabez, R.J. Huxtable, Effects of the taurine transporter antagonist, guanidinosephonate sodium, and beta-alanine on the morphology of rat retina, J. Neurosci. Res. 9 (1983) 135–143.
[8] M.S. Starr, M.J. Voaden, The uptake, metabolism and release of 14C-taurine by rat retina in vitro, Vis. Res. 12 (1972) 1261–1269.
[9] C. Tiruppathi, M. Brandsch, Y. Miyamoto, V. Ganapathy, F.H. Leibach, Constitutive expression of the taurine transporter in a human colon carcinoma cell line, Am. J. Physiol. 263 (1992) G625–G631.
[10] Z. Zang, et al., Taurine supplementation reduces oxidative stress and protects the liver in an iron-overload murine model, Mol. Med. Rep. 10 (2014) 2255–2262.
[11] D. Dawson Jr., S. Liu, B. Eppler, T. Patterson, Effects of dietary taurine supplementation or deprivation in aged male Fischer 344 rats, Mech. Ageing Dev. 107 (1999) 73–91.
[12] M. Ansar, et al., Taurine treatment of retinal degeneration and cardiomyopathy in a congenital amniotic family with SLC6A6 taurine transporter deficiency, Hum. Mol. Genet. 20 (2011) 618–623.
[13] O. Quesada, A. Picones, H. Pasantes-Morales, Effect of light deprivation on the ERG responses of taurine-deficient rats, Exp. Eye Res. 46 (1988) 13–20.
[14] K.N. Rasmussen, C. Lagunas, J. Plum, R. Holm, C.U. Nielsen, Interaction of GABA-A receptors with retinal photoreceptors in vitro, Invest. Ophthalmol. Vis. Sci. 57 (2016) 4692–4703.
[15] A. Adkekeye, M. Haeri, E. Solostoe, B.E. Knox, Ablation of the proapoptotic gene CHOP or Ask 1 does not prevent or delay loss of visual function in a P23H transgenic mouse model of retinitis pigmentosa, PLoS One 9 (2014), e83871.
[16] T.P. Dryja, et al., A point mutation of the rhodopsin gene in one form of retinitis pigmentosa, Nature 343 (1990) 364–366.
[17] X. Liu, P. Garriga, H.G. Khorana, Structure and function in rhodopsin: correct folding and misfolding in two point mutants in the intradiscal domain of rhodopsin identified in retinitis pigmentosa, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 4554–4559.
[18] E.L. Berson, K.C. Hayes, A.R. Rabin, S.Y. Schmidt, G. Watson, Retinal degeneration in cats fed casein. II. Supplementation with methionine, cysteine, or taurine, Invest. Ophthalmol. 15 (1976) 52–66.
[19] S.Y. Schmidt, L.I. Berson, K.C. Hayes, Retinal degeneration in the taurine-deficient cat, Trans. Sect. Ophthalmol. Am. Acad. Ophthalmol. Otolaryng. 81 (1976) OP678–693.
[20] J. Tsybovsky, R.S. Molday, K. Palczewski, The ATP-binding cassette transporter ABCA4: structural and functional properties and role in retinal disease, Adv. Exp. Med. Biol. 703 (2010) 105–125.
