Effect of light exposure on the accumulation and depletion of retinyl ester in the chicken retina

Elia T. Villazana-Espinoza, Andrea L. Hatch, Andrew T.C. Tsin*

Department of Biology, The University of Texas at San Antonio, 6900 N Loop 1604 W, San Antonio, TX 78249, USA

Received 27 February 2006; accepted in revised form 18 April 2006
Available online 15 June 2006

Abstract

A previous study has shown that in the cone-rich chicken retina, 11-cis retinyl ester increases with light exposure and decreases in subsequent dark adaptation. The purpose of this research is to study how light exposure (intensity and duration) determine the rate of accumulation and utilization as well as the size of this 11-cis retinyl ester pool in the chicken retina. Chickens were dark-adapted overnight before exposure to different light intensities and durations. Animals were sampled at regular time intervals. 11-cis retinal and 11-cis retinyl ester were extracted from the retina and analyzed by HPLC. An increase in light intensity from 1000 and 2000 Lux (for a 20 min exposure) increased the amount of 11-cis retinyl ester from 0.38 to 0.75 nmol/mg. An increase in the duration of light exposure from 10 to 20 min (at 2000 Lux) also increased the amount of 11-cis retinyl ester in the chicken retina (from 0.37 to 0.75 nmol/mg). This 11-cis retinyl ester pool in the chicken retina was rapidly reduced to baseline level (~0.20 nmol/mg) upon dark adaptation. The rate of accumulation of 11-cis retinyl ester was dependent on light intensity and duration of exposure and the maximum rate was ~0.03 nmol/mg/min. In comparison, dark adaptation was associated with a significantly higher rate of 11-cis retinyl ester depletion (~0.05), indicating that light exposure and dark adaptation were associated with different biochemical steps of retinoid storage and utilization. Results from this study are the first to show that the size of the 11-cis retinyl ester pool, as well as the rate of its accumulation and depletion in the cone-rich chicken retina, are determined by the intensity and duration of light exposure. These data support the suggestion that a light-driven cone cycle exists in the chicken retina.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: retinoids; retina; light and dark adaptation; visual cycle; cones; chicken

1. Introduction

The rod visual cycle and the role that retinoids play in photopigment regeneration have been extensively characterized (Crouch et al., 1996; Lamb and Pugh, 2004; McBee et al., 2001; Rando, 2001; Saari, 2000). However, evidence for a proposed cone visual cycle has only been recently accrued (Bustamante et al., 1995; Gollapalli and Rando, 2003; Mata et al., 2002, 2005; Trevino et al., 2005). Cone pigments can spontaneously regenerate in isolated frog retina (Goldstein, 1967; Hood and Hock, 1973) without the support of adjacent retinal pigment epithelium (RPE, which is required for rod pigment regeneration) (Crouch et al., 1996; Saari, 2000). Cone but not rod sensitivity was recovered upon the addition of 11-cis retinol in isolated, bleached salamander photoreceptors (Jones et al., 1989). Rodriguez and Tsin (1989) reported that cone-dominated chicken retina, compared to rod-dominated cow and frog retinas, stores a high amount of 11-cis retinyl ester. Subsequently, Das et al. (1992) confirmed that cone-dominated animals stored higher levels of 11-cis retinyl esters in their retinas. They also reported that cultured Muller cells from the chicken retina convert labeled all-trans retinol to 11-cis retinol and 11-cis retinyl palmitate. Based on the finding that the cone-dominated chicken retina possesses significant 11-cis retinyl ester hydrolase activity (to release retinol from retinyl ester for pigment regeneration), Bustamante et al. first suggested that a visual cycle may reside in the retina of the cone-dominated chicken eye (Bustamante et al., 1995).
Mata et al. (2002) characterized the activity of three novel visual cycle enzymes (retinol isomerase, retinol dehydrogenase, and retinyl ester synthase) in retinal and RPE homogenates prepared from chicken and ground squirrel. Their conclusion that a novel retinol isomerase catalyzed retinol isomerization at the alcohol oxidation level was subsequently challenged by results from experiments from another laboratory (Gollapalli and Rando, 2003) that showed the involvement of all-trans retinyl ester and isomerohydrolase in the isomerization of retinoids in the chicken retina. Recently, Mata et al. (2005) reported additional data in support of a functional retinol isomerase and a retinyl ester synthase (or "isomeromerase"), which work in concert to synthesize 11-cis retinoids in the chicken retina.

Direct, in-vivo evidence to demonstrate the existence of a visual cycle to support cone visual pigment regeneration was recently obtained from the chicken retina (Trevino et al., 2005). Light exposure induced a steady accumulation of 11-cis retinyl esters in the retina and subsequent dark adaptation rapidly returned 11-cis retinyl ester to baseline level within minutes. This accumulation and depletion of 11-cis retinyl ester in the retina were attributable to the retinoid chromophore released from bleaching and regeneration of cone pigments, based on quantitative, reciprocal changes of 11-cis isomerization (in the retina) in response to different light intensities. Hence, it is not clear how light exposure (intensity and duration) determines the rate of accumulation and utilization, as well as the size of this pool of 11-cis retinyl ester in the retina.

In the present study, we have carried out additional experiments to further understand this light-induced accumulation and depletion of 11-cis retinyl ester in the chicken retina by measuring the change in 11-cis retinyl ester in response to different durations and intensities of light exposure. Our results confirmed that light exposure led to the accumulation of retinyl ester while the depletion of this retinyl ester pool was initiated by subsequent dark adaptation. Furthermore, this study is the first to provide experimental data to show that the size of this 11-cis retinyl ester pool increased with light intensity and duration of exposure. In addition, our data also revealed a significant difference between the rates at which this pool of 11-cis retinyl ester was accumulated and depleted in the retina, indicating different biochemical steps for retinoid chromophore storage and utilization. Overall, results of the present study provide strong experimental evidence to show that ambient light induces the accumulation and utilization of retinyl esters in the cone-rich chicken retina in support of a light-driven retinoid cycle for cone pigment regeneration.

2. Materials and methods

In accordance with guidelines from the National Institute of Health (NIH), all experiments were carried out in adherence to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Our research protocol was also approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at San Antonio (UTSA). Young chickens (Gallus domesticus, ~60 gm) were purchased from Producer’s Co-op in Seguin, Texas and maintained at UTSA Bioscience animal housing facility.

2.1. Change in 11-cis-retinal and 11-cis-retinyl ester (in the retina) in response to different light intensities

Young chickens were dark-adapted overnight. For light adaptation, they were exposed to bright illumination at three different light intensities (one 90 W halogen bulb or 1000 Lux, two 90 W bulbs or 2000 Lux and three 90 W bulbs or 3000 Lux) at 45 cm distance, for 20 min before sampling. Light intensity was recorded with a commercially available light meter (Sekonic Handy Lumi model 246). Chickens were removed from their cage and placed in an induction chamber. They were then anesthetized by CO2 and decapitated before eye enucleation. Retinas were removed, separated from RPE, and placed in test tubes containing 1 ml 10 mM Tris pH 7.5 buffer and immediately immersed in dry ice and acetone. Material remained frozen at −85 °C before retinoid extraction and HPLC analyses. [Note: Based on results from an earlier study (Trevino et al., 2005), retinoids of the rod cycle accumulate in the chicken RPE which is not the focus of the present study. Therefore, retinoids in the RPE were not measured in the present study]. Two animals were used per treatment group and left and right retinas were analyzed separately. Five experiments were conducted on the effect of light intensities.

2.2. Change in 11-cis-retinyl ester (in the retina) in response to different durations of light exposure and during subsequent darkness

Young chickens were dark adapted overnight. Then they were exposed to two 90 W halogen bulbs or 2000 Lux for three different durations: 10, 20 and 40 min, before dark adaptation up to a maximum period of 40 min. Animals were sampled at 10 or 20 min intervals during the light and dark phases as detailed in Section 3. Animals were sacrificed and sampled as indicated above. Two animals were used per time point in each of the three experiments (10, 20 and 40 min light exposures) and left and right retinas were analyzed separately. Each experiment was repeated twice.

2.3. Extraction and quantitation of retinoids

To extract retinyl esters, retinal samples were homogenized in a final volume of 2 ml of 10 mM Tris pH 7.5 buffer. Three 10 μl aliquots were removed for protein determination (3.10 ± 0.11 mg per retina, n = 10, and 0.90 ± 0.14 mg per RPE, n = 10) and retinyl esters in the remaining sample was extracted twice by ethanol and hexane. Retinoid extracts were saponified in strong base (0.33 M ethanolic KOH solution) and the retinol products were analyzed by HPLC (717 plus Waters auto sampler, Waters 515 pump and photodiode array detector) eluted with 10% dioxane/hexane at 2 ml/min. To extract retinals,
we employed both the formaldehyde (Suzuki et al., 1986) and the hydroxylamine (Groenendijk et al., 1980) methods and retinals (or syn/anti retinyl oximes) were eluted from the HPLC with 1% dioxane/hexane at 2 ml/min. Retinoids were identified by relative retention time and by on-line photodiode array scans. Quantification was carried out by standard curves previously established with authentic standards (Waters Millennium Software).

3. Results

To determine the efficacy of the formaldehyde method, chicken retinas were sampled before and after exposure to 2000 Lux for 20 min. A fully dark-adapted retina had 0.23 nmol/mg of 11-cis retinal (Fig. 1A, upper diagram). After 20 min of light exposure, this amount was reduced to 0.06 nmol/mg (Fig. 1A, lower diagram). Correspondingly, 11-cis retinyl ester extracted from a dark-adapted retina was minimal (0.24 nmol/mg; see Fig. 1B, upper diagram). Upon light exposure, the amount of 11-cis retinyl ester increased significantly to 0.75 nmol/mg (Fig. 1B, lower diagram). Similar results were obtained with the hydroxylamine method for extraction and analyses of retinal.

To examine the effects of light intensity, retinas of dark-adapted chickens were sampled before and after 20 min exposure to 1000, 2000 and 3000 Lux. Dark-adapted retinas contained 0.23 nmol 11-cis retinal/mg retinal protein (Fig. 2A). Most of the photopigments were bleached by light-exposure (about 70% reduction in 11-cis retinal to 0.06–0.08 nmol/mg) (Fig. 2A) while a concomitant increase in 11-cis retinyl ester was noted in the retina (Fig. 2B). Exposure to 1000 Lux for 20 min resulted in an increase of 0.14 nmol/mg 11-cis retinyl ester (i.e. 0.38–0.24 nmol/mg; Fig. 2B). Exposure to 2000 Lux resulted in a significantly higher level of 11-cis retinyl ester accumulation of 0.51 nmol/mg (i.e. 0.75–0.24 nmol/mg; Fig. 2B) whereas exposure to 3000 Lux resulted in only a small increment of 11-cis retinyl ester to 0.54 nmol/mg (i.e. 0.78–0.24 Fig. 2B).

The duration of light exposure also significantly affected the amount of 11-cis retinyl ester in the retina as evidenced by sampling before, during and after exposure to 2000 Lux illumination. After 10 min of light exposure at 2000 Lux, the chicken retina had accumulated 0.14 nmol/mg 11-cis retinyl ester/mg (i.e. 0.38–0.24 nmol/mg; Fig. 2B). Exposure to 2000 Lux resulted in a significantly higher level of 11-cis retinyl ester of 0.51 nmol/mg (0.75–0.18 nmol/mg, Fig. 2B). Exposure of 40 min also resulted in an increase in the accumulation of 11-cis retinyl ester of 0.73 nmol/mg (0.90–0.17 nmol/mg; Fig. 3C).

The rate of accumulation and depletion of 11-cis retinyl ester are summarized in Table 1. The maximum rate of accumulation in all experiments was estimated to be 0.03 nmol retinyl
ester per mg per min whereas the maximum rate of utilization (derived from data in Fig. 3) was about 2 times higher (0.05 nmol/mg/min).

4. Discussion

In a recent publication, we reported that the chicken retina contains an active retinoid cycle (i.e. accumulation and depletion of 11-cis retinyl esters) in support of the bleaching and regeneration of cone pigment (Trevino et al., 2005). However, it was not determined how light intensity and duration of exposure induced this accumulation and depletion of retinyl esters in the chicken retina. Data from the present study confirm that light bleached visual pigments (Fig. 1, reduction in 11-cis retinal) with a corresponding increase (or accumulation) of 11-cis retinyl esters in the retina (Fig. 1, increase in retinyl ester). Subsequent dark adaptation resulted in the depletion of this retinyl ester pool (see Figs. 3A–C), suggesting utilization of retinoid for pigment regeneration (Trevino et al., 2005). Therefore, our results strongly support the suggestion that this retinoid cycle in the retina is light-driven.

Fig. 2. Change in 11-cis retinal (A); and 11-cis retinyl esters (B) in the chicken retina in response to different light intensities. Animals were dark-adapted overnight before exposed to different light intensities for 20 min. Mean ± S.E.M. were calculated from results of five experiments. For each of the five experiments, retinoid extracts from individual retina were analyzed by HPLC and results from four retinas (2 animals per treatment group) were averaged (n = 4).

Fig. 3. Change in 11-cis retinyl esters in the retina in response to 10 min (A); 20 min (B); and 40 min (C) of light exposure and then dark adaptation. Animals were dark-adapted (DA) overnight and then exposed to light and sampled at indicated time points. Mean ± S.E.M. were calculated from results of three experiments. For each of the three experiments, retinoid extracts from individual retina were analyzed by HPLC and results from four retinas (2 animals per time point) were averaged (n = 4). The total number of animals for each experiment in Figs. 3A–C were 8, 10 and 12 respectively.

Furthermore, results from the present study are the first to show that this amount of 11-cis retinyl ester in the retina was determined by the light intensity and duration of exposure. Data in Fig. 2 shows that light exposure at 2000 Lux (for 20 min)
resulted in the accumulation of about 0.51 nmol retinyl ester/mg retinal protein (see Section 3 and Fig. 2B). Correspondingly, light intensity at 2000 Lux bleached about 74% of the visual pigments (from 0.23 to 0.06 nmol 11-cis retinal/mg; Fig. 2A). Based on the fast kinetics of cone pigment bleaching and regeneration [see Fig. 3, (Trevino et al., 2005)], 11-cis retinal reduction took place within the first 5 min of light exposure], the rate of pigment bleaching in the retina is estimated minimally to be 0.03 nmol/mg/min (i.e. 0.23–0.06 = 0.17 nmol/mg in 5 min). Assuming that this rate of bleaching was sustained in 20 min, about 0.60 nmol/mg of chromophore (all-trans retinal) was produced (0.03 nmol/mg/min × 20 min) from light exposure. In comparison, 0.51 nmol/mg of retinyl ester was accumulated during this exposure period, suggesting that this retinyl ester pool was likely derived from retinal chromophore of bleached visual pigments. (Light intensity at 3000 Lux bleached about the same % of photopigments and resulted in a similar amount of 11-cis retinyl ester.) It is also possible that light activates a process in the eye to supply additional retinoids from the retinal pigment epithelium (RPE) for pigment bleaching and regeneration during this period of light exposure. The exact mechanism of this RPE/retina retinoid exchange remains to be studied.

About 0.14 nmol/mg of retinyl ester was accumulated in the retina exposed to 1000 Lux (for 20 min) which bleached 65% of visual pigments. This amount of retinyl ester was much lower than 0.51 nmol/mg of retinyl ester associated with 2000 Lux which bleached a similar portion (i.e. 74%) of visual pigments. This is explicable by a significantly lower rate of bleaching of ~0.01 nmol/mg/min associated with the lower light intensity of 1000 Lux [i.e. in comparison to 0.03 nmol/mg/min at 2000 Lux; (Lamb and Pugh, 2004)]. This bleaching rate resulted in a significantly lower amount of retinoid chromophore released from bleached visual pigments (i.e. 0.01 nmol/mg/min × 20 min = 0.20 nmol/mg).

Consistent with our previous report, we did not observe a significant accumulation of all-trans retinal, all-trans retinol and 11-cis retinol in the retina (Trevino et al., 2005). This suggests that in the cone-dominated chicken retina, all-trans retinoids may be rapidly converted to 11-cis retinol and then esterified to become 11-cis retinyl ester. The location of 11-cis retinyl ester in the retina is currently being investigated in our laboratory (Muniz et al., 2006). It is possible that 11-cis retinyl ester may be stored in Muller cells (Das et al., 1992; Mata et al., 2002) and that it is associated with the plasma membrane (Imanishi et al., 2004; Mata et al., 1998). The exact mechanism of cis retinoid synthesis from all-trans retinol in the chicken retina is also under investigation at this time (Villazana-Espinoza et al., 2006).

Fig. 2B also indicates that the increase of light intensity from 1000 to 2000 Lux resulted in a significant increase in the amount of retinyl ester accumulated in the retina. Thus the size of the retinyl ester pool is dependent on light intensity. However, increase of light intensity to 3000 Lux did not result in a large increment in the accumulation of retinyl ester, suggesting that this pool of 11-cis retinyl ester may saturate at a certain level (i.e. close to 0.8–0.9 nmol/mg; see also Section 4 below on the duration of light exposure). The mechanism of light activation of 11-cis retinoid synthesis in the retina is not known at this time. However, it has been reported that a non-visual opsin, RGR (Retinal G protein-Coupled Receptor), located in the RPE and Muller cells, synthesizes 11-cis retinal from all-trans retinal when irradiated with 470 monochromatic light (Hao et al., 2000). This provides a possible mechanism of light-induced 11-cis retinoid synthesis in the retina.

Fig. 3 shows the accumulation and depletion of retinyl esters in the retina during light exposure (at 2000 Lux) and subsequent dark adaptation. The data in Fig. 3B are consistent with those in Fig. 2B that 0.75 nmol 11-cis retinyl ester/mg accumulated in the retina (exposed to 2000 Lux for 20 min). Comparison of Figs. 3A,B show that the amount of 11-cis retinyl ester in the retina was significantly reduced (from 0.57 to 0.19 nmol/mg; see Section 3 and Fig. 3A) when the duration of light exposure was changed from 20 to 10 min. This is consistent with our earlier suggestion that the amount of 11-cis retinyl ester accumulated in the retina is dependent on the rate of bleaching and the duration of exposure. However, the change from 20 to 40 min of light exposure induced a smaller increase of accumulation of this 11-cis retinyl ester pool from 0.57 to 0.73 nmol/mg (see Section 3 and Fig. 3C). It is possible that this retinoid pool may saturate when it approaches 0.8–0.9 nmol/mg (see Section 4 above). At present, it is not known if this saturation is the result of a physical limit of the size of the storage or the result of an equilibrium existing between retinoid accumulation and utilization.

Results from Fig. 3 also show that dark adaptation (subsequent to light exposure) induced a rapid decrease in the amount of retinyl ester in the retina. Figs. 3A,B show that almost the entire amount of accumulated retinyl ester in the retina was depleted in the first 10 min of dark adaptation. Based on this rate of reduction of 11-cis retinyl ester, it is very likely that these retinoids were depleted to supply visual chromophore for pigment regeneration. [Although we did not measure the recovery of 11-cis retinol in the present study, our previous study has

Table 1

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Rate of accumulation (light)</th>
<th>Rate of depletion (dark)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 Lux for 20 min</td>
<td>+0.01</td>
<td></td>
</tr>
<tr>
<td>2000 Lux for 20 min</td>
<td>+0.03</td>
<td></td>
</tr>
<tr>
<td>3000 Lux for 20 min</td>
<td>+0.03</td>
<td></td>
</tr>
<tr>
<td>2000 Lux for 10 min; dark for 10, 20 min (Fig. 3A)</td>
<td>+0.02, +0.03, +0.03, +0.03, +0.03</td>
<td></td>
</tr>
<tr>
<td>2000 Lux for 10, 20, 40 min; dark for 20, 40 min (Fig. 3B)</td>
<td>+0.02, +0.03, +0.04, +0.02</td>
<td></td>
</tr>
<tr>
<td>Maximum rate</td>
<td>+0.03, +0.05</td>
<td></td>
</tr>
</tbody>
</table>

Data in this table are calculated from those presented in Figs. 2B and 3A–C based on the following equation: change in the amount of retinyl ester during light or dark treatment (in nmol/mg) divided by the duration of light or dark treatment (in minutes).
provided clear experimental evidence to show that the level of 11-cis retinal was fully restored rapidly while 11-cis retinyl ester depleted during this dark adaptation (Trevino et al., 2005).

Dark adaptation subsequent to light exposure returned the level of 11-cis retinyl ester to baseline much more rapidly than it had accumulated in light (Figs. 3A–C). This suggests that the rate of utilization of retinyl ester far exceeded the rate of accumulation. Quantitative comparisons show that the maximum rate of 11-cis retinyl ester accumulation was 0.03 nmol/mg/min whereas the maximum depletion rate was approximately 2 times (0.05 nmol/mg/min, Table 1). Based on results in Figs. 3A,B, the level of 11-cis retinyl ester was returned to baseline within 10 min of dark adaptation. If this had taken place in the experiment described in Fig. 3C, the maximum rate of depletion of 11-cis retinyl ester after 40 min of exposure would have exceeded 0.06 nmol/mm/min (i.e. 0.9–0.2 = 0.7 nmol/mg in 10 min, see Fig. 3C, this data point was not collected in this experiment). The maximum accumulation rate of 11-cis retinyl ester reported in the present study is somewhat lower than those (rates of enzymatic catalysis) obtained from in-vitro assays [ARAT activity in Muller cell membrane; 0.2 nmol/min/mg; (Muniz et al., 2006); isomerization activity in chicken retinal and RPE membranes 0.40 nmol/min/mg, Fig. 3C (Mata et al., 2002) and in chicken retinal membrane, \( V_{\text{max}} = 0.82 \text{ nmol/min/mg} \), Table 1 (Mata et al., 2005)]. The higher rate of depletion of 11-cis retinyl ester in the retina is consistent with a high chromophore demand due to the fast rate of cone pigment regeneration (Lamb and Pugh, 2004). It is possible that during light exposure, retinyl ester accumulation and depletion take place simultaneously whereas only retinyl ester depletion takes place in the dark. This provides an explanation as to why the rate of retinyl ester “accumulation” (which is a net rate of increase retinyl ester for storage and decrease retinyl ester for utilization) observed in the present study is lower than that of retinyl ester “depletion” (decrease retinyl ester for utilization only). Nevertheless, 11-cis retinyl ester accumulation must associate with a set of visual cycle enzymes (isomerase, ester synthase) which are completely different than those visual cycle enzymes used for the utilization of 11-cis retinyl esters for visual pigment regeneration (ester hydrolase and retinol dehydrogenase). Thus distinct biochemical pathways of retinyl ester accumulation and depletion also obviate different rates of retinyl ester accumulation and utilization in the chicken retina. The detailed mechanisms of these biochemical pathways remain to be investigated.

Acknowledgements

We thank Drs. Nathan Mata, Don Allen and Simon Trevino for their critical review of this manuscript. This research was supported by grants from the NIH (GM 08194) and the Kronkosky Charitable Foundation.

References


