Choroidal Retinoic Acid Synthesis: A Possible Mediator between Refractive Error and Compensatory Eye Growth

JAMES R. MERTZ AND JOSH WALLMAN

Department of Biology, City College, City University of New York, 138th Street and Convent Avenue, New York, NY 10031, U.S.A.

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Research over the past two decades has shown that the growth of young eyes is guided by vision. If near- or far-sightedness is artificially imposed by spectacle lenses, eyes of primates and chicks compensate by changing their rate of elongation, thereby growing back to the pre-lens optical condition. Little is known about what chemical signals might mediate between visual effects on the retina and alterations of eye growth. We present five findings that point to choroidal retinoic acid possibly being such a mediator.

First, the chick choroid can convert retinol into all-trans-retinoic acid at the rate of 11 ± 3 pmoles mg protein⁻¹ hr⁻¹, compared to 1.3 ± 0.3 for retina/RPE and no conversion for sclera. Second, those visual conditions that cause increased rates of ocular elongation (diffusers or negative lens wear) produce a sharp decrease in all-trans-retinoic acid synthesis to levels barely detectable with our assay. In contrast, visual conditions which result in decreased rates of ocular elongation (recovery from diffusers or positive lens wear) produce a four- to five-fold increase in the formation of all-trans-retinoic acid. Third, the choroidal retinoic acid is found bound to a 28–32 kD protein. Fourth, a large fraction of the choroidal retinoic acid synthesized in culture is found in a nucleus-enriched fraction of sclera. Finally, application of retinoic acid to cultured sclera at physiological concentrations produced an inhibition of proteoglycan formation (as assessed by measuring sulfate incorporation) with a EC₅₀ of 8 × 10⁻⁸ M. These results show that the synthesis of choroidal retinoic acid is modulated by those visual manipulations that influence ocular elongation and that this retinoic acid may reach the sclera in concentrations adequate to modulate scleral proteoglycan formation.

Key words: sclera; choroid; chick; myopia; hyperopia; proteoglycan.

1. Introduction

The growth of the eye is regulated not only by the developmental mechanisms common to all organs, but also by feedback supplied by the visual input. This regulation by visual feedback is demonstrated by the rapid compensation that eyes of chicks and monkeys show when hyperopia or myopia is imposed on them by spectacle lenses (Irving, Sivak and Callender, 1992; Schaeffel, Glasser and Howland, 1988; Smith and Hung, 1999; Wildsoet and Wallman, 1995). It is likely, at least in the case of chicks, that this compensation is effected in part by modulation of the growth of the sclera causing changes in the length of the eye (Marzani and Wallman, 1997; Nickla, Wildsoet and Wallman, 1997). Another influence of the retina on eye growth is shown by the ocular elongation and myopia provoked by form-deprivation in all species studied (Troilo and Judge, 1993; Wallman, Turkel and Trachtman, 1978; Marshall-Tootle, 1989; Wiesel and Raviola, 1977). Although it is unclear at present whether the same mechanisms underlie this growth change as that provoked by wearing spectacle lenses, both involve modulation of scleral growth (Rada and Matthews, 1994) and both appear to be largely local to the region of retina experiencing the altered vision (Diether and Schaeffel, 1997; Wallman et al., 1995). Children tend to develop myopia during the school years, with the incidence of myopia being associated with the amount of reading or other near-work (Saw et al., 1996; Zylbermann, Landsau and Berson, 1993). As reading imposes a slight hyperopic defocus on the eye (accommodation does not entirely eliminate it) (Rouse, Hutter and Shiftlett, 1984; Schaeffel, Weiss and Seidel, 1999), interest has been drawn to the possibility that the myopia attendant to heavy reading is a normal compensatory response to this hyperopia and thus is much like the spectacle lens compensation shown by animals.

Much work has gone into identifying what chemical signals might participate in the retina’s influence on eye growth. Strong evidence points to acetylcholine, in that antagonists to muscarinic acetylcholine receptors reduce both form-deprivation myopia and the progression of clinical myopia in children (Kennedy, 1995; McBrien, Moghaddam and Reeder, 1993; Stone, Lin and Laties, 1991), although it is quite unclear which of the several ocular tissues on which it acts is the relevant one (Fischer et al., 1998; Lind et al., 1998). Changes in retinal dopamine are also clearly correlated with form-deprivation myopia.
(Stone et al., 1989), which can be ameliorated in chicks and monkeys by dopamine agonists (Iuvone et al., 1991). Retinal dopamine may also be involved with the defocus caused by spectacle lenses (Guo et al., 1995; Schaeffel et al., 1995), but the correlation is far from perfect (Boelen et al., 1999; Schwahn and Schaeffel, 1997). Finally, glauconergeric amacrine cells show opposite changes in ZENK expression in chick eyes wearing positive and negative spectacle lenses (Fischer et al., 1999).

Whatever retinal chemical signals might be involved, a major puzzle is how the retinal signals could cross the choroid, a tissue layer with high blood flow and extensive fenestrated capillaries. We propose that no retinal signal need cross the choroid. Instead the retina may signal the choroid to produce retinoic acid, which in turn influences the growth of the sclera.

Retinoic acid is an important signaling molecule in the developing eye. In mice with knock-outs of the retinoic acid receptors, the eyes are extremely small with gross morphological defects in choroid and sclera and retinal dysplasia (Gronodona et al., 1996). In tissue culture, retinoic acid is required for the survival and differentiation of embryonic photoreceptor cells (Kelley, Turner and Reh, 1994; Stenkamp, Gregory and Adler, 1995). Furthermore, in both mice and chicks, dorsal-ventral patterning of the retina is determined by gradients of retinoic acid (McCaffery et al., 1992; Mey, McCaffery and Drager, 1997). Finally, in postnatal animals retinoic acid modulates the expression of growth factors such as TGFβ (MacDonald et al., 1995) and causes anatomical and physiological light adaptation in carp retinas (Weiler et al., 1998).

In this paper we show a pattern of results which argue that it is plausible that the choroid synthesizes and releases retinoic acid at a level determined by the visual circumstances of the retina; this retinoic acid is transported to the sclera and there inhibits extra-cellular matrix synthesis, thereby influencing ocular elongation. In brief, our evidence is (a) the choroid produces massive amounts of retinoic acid, the levels of which are strongly affected by spectacle lenses or by form-deprivation with diffusers; (b) the choroidal retinoic acid is released into medium where it is found associated with specific proteins; (c) scleral tissue accumulates retinoic acid, but synthesizes little or none; (d) retinoic acid strongly inhibits the synthesis of proteoglycans by the sclera in vitro at physiological concentrations.

2. Materials and Methods

Visual Manipulations

To deprive eyes of form-vision, white translucent vinyl diffusers were attached to the feathers around one eye of 2-day-old chicks for 14 days. This results in myopia and ocular elongation. Other chicks wore a diffuser for 11 days, after which the diffuser was removed, and the eye was allowed to recover for 3 days. To impose myopia or hyperopia with spectacle lenses, chicks 6–7 days old wore a +15 D or a −15 D lens, respectively, over one eye for 24 hr (details in Wildsoet and Wallman, 1995).

Determination of in vitro Synthesis of Retinoic Acid

After pentobarbital killing, eyes were removed, placed on a bed of ice, hemisected at the ora serrata, and the vitreous removed. An 8 mm punch, taken from the posterior pole of each eye, was placed in Dulbecco’s Modified Eagle’s Medium (DMEM), and the retina and most of the retinal pigment epithelium (RPE) were removed. After any remaining RPE cells were brushed off, the choroid was peeled from the sclera. The isolated choroid, sclera, and retina plus RPE were each kept in ice-cold DMEM prior to culture. Tissues were incubated in 0.5 ml of the defined medium N2 of Bottenstein and Sato (1979) containing 1 µCi ml⁻¹ all-trans-retinol ([20-methyl-³H] Dupont NEN Research Products, Boston, MA, U.S.A.) for 2 hr at 37°C, following which media and tissues were stored in separate amber tubes at −80°C until analysis. Tissues were homogenized in 0.5 ml PBS, an aliquot was removed for protein determination by the Bradford method (Biorad, Hercules, CA, U.S.A.), 0.6 ml of isopropyl alcohol (containing 100 ng of unlabeled 9-cis-, 13-cis- and all-trans-retinoic acid as carriers to permit identification of the HPLC peaks of these retinoids for isolation and counting) was added, vortexed for 5 min, centrifuged at 10000 g for 10 min and the supernatant mixed with 2% acetic acid solution to a final concentration of 25% isopropyl alcohol. The resulting solution was applied to a solid phase extraction column (100 mg C8, Baxter Scientific, Chicago, IL, U.S.A.), which had been equilibrated in 1% acetic acid solution, washed with 2 ml of 1% acetic acid in water: methanol 60:40, then washed with 100 µl of methanol and air dried for 1 min. Retinoic acid was eluted from the column with 2 × 300 µl of ethanol: methanol 75:25, dried with a stream of N2 gas and dissolved in 100 µl of 1% acetic acid in water: acetonitrile 60:40 for HPLC injection. Isomers of retinoic acid were separated using a reverse phase column (ODS2, 25 cm × 3.1 mm, 5 µm, with an additional C18 guard column; Keystone Scientific, Keystone, PA, U.S.A.) and isocratic elution at 65°C at a flow rate of 0.6 ml min⁻¹. The mobile phase was acetonitrile:1% acetic acid in water 65:35. The absorbance of the eluant was monitored at 350 and 325 nm utilizing a diode array detector (Waters Instruments, Medford, MA, U.S.A.). Each retinoid peak was collected manually and the amount of radioactive retinoid was determined with a liquid scintillation counter (Beckman Instruments, LS 1800, Columbia, MD, U.S.A.). To estimate background radioactivity, samples equivalent in volume to those of the retinoic acid peaks were collected prior to and after the retinoic
acid peaks. For each retinoic acid peak, the background radioactivity was subtracted, and the net counts were corrected for percent recovery as determined by the retinoid standard for that peak. The resulting counts were converted to pmol of retinoic acid based on the specific activity of retinol, which was determined by collecting the retinol HPLC peak from tissue samples and determining its mass by absorbance at 325 nm and its total radioactivity. The identification of the radioactive retinoic acid isomers was confirmed by normal phase HPLC, using two columns in tandem array (Spherical Silica 7.5 cm x 4.6 mm, 5 µm, Waters Instruments, Medford, MA, U.S.A. and a 3 µm Supelcosil column, 7.5 cm x 4.6 mm, Sigma, St. Louis, MO, U.S.A.), with an isocratic elution at a flow rate of 1.8 ml min⁻¹. The mobile phase was acetic acid: acetonitrile:hexane 0:1:04:99:5. All culture experiments, retinoic acid extractions and HPLC analyses were performed under either dim red light or monochromatic light of 589 nm.

Measurement of the in vivo Retinoic Acid Levels in Sclera

Scleral tissue from five eyes (7 mm punches) was pooled and homogenized with a Polytron homogenizer (Brinkmann, Westbury, NY, U.S.A.) in 2 ml of water. Lipids were extracted by adding 4 ml of chloroform: methanol 2:1, the sample was vortexed for 1 min, and an additional 2 ml of chloroform was added. The sample was centrifuged at 2000 g for 15 min and the chloroform layer was removed and reduced to 1 ml by evaporation with a gentle stream of N₂ gas. The acidic lipids were isolated from the chloroform by solid phase extraction using an aminopropyl-bonded silica gel column, and retinoic acid levels were determined by normal phase HPLC using a Waters model 2487 Dual Wavelength Absorbance Detector or Waters Diode array detector model 996 (Kurlandsky et al., 1995).

SDS Gel Electrophoresis of Binding Proteins

To examine whether the retinoic acid released into the medium was associated with specific proteins, we made choroid-conditioned medium from ten choroids of eyes recovering from form-deprivation myopia by incubating them with tritiated all-trans-retinol for 2 hr in N2 medium lacking catalase or conalbumin (removed so that the only proteins in the conditioned medium were from the choroidal tissue). The resulting medium was divided into four 2.5 ml aliquots and incubated for 1 hr with either 13-cis- or all-trans-retinoic acid or a mixture of all-trans- and 9-cis-retinoic acid, in all cases at a concentration of 100 ng ml⁻¹, or with 10 µl of carrier (ethanol). Each aliquot was concentrated to 100 µl with an Amicon Centriprep-10 filter at 4°C, and the concentrated conditioned medium was UV-irradiated to cross-link the retinoic acid to carrier proteins. To determine which proteins were covalently labeled with retinoic acid, the cross-linked proteins were fractionated by SDS gel electrophoresis (Bernstein et al., 1995), the gel was sliced into 2 mm pieces, digested with 30% hydrogen peroxide (0.5 ml slice⁻¹) overnight at 37°C and the radioactivity in each slice measured by scintillation counting.

Inhibition of Proteoglycan Synthesis

To assess proteoglycan synthesis, we measured the incorporation of precursor into glycosaminoglycans (GAGs). Scleral punches, 8 mm in diameter, from week-old chicks were incubated with various concentrations of retinoic acid for 22 hr, pulsed with Na₂¹³SO₄ for 2 hr, digested with protease K, and the GAGs precipitated and counted as previously described (Marzani and Wallman, 1997). Scleral punches from untreated fellow eyes were incubated under the same conditions with vehicle (ethanol). Inhibition was assessed by dividing the synthetic rate in the experimental eye by that in the untreated fellow eye of each animal.

3. Results

The Choroid, as well as the Retina, Converts Retinol to Retinoic Acid

By separately incubating the tissue layers of the chick posterior eye wall with tritiated all-trans-retinol and analysing the metabolic products using HPLC, we found that under our culture conditions the choroid produces 20-fold more all-trans-retinoic acid (per milligram protein) than does the retina plus retinal pigment epithelium, established sources of ocular retinoic acid (Table I) (Edwards et al., 1992; McCaffery, Mey and Drager, 1996; Mey et al., 1997). These synthetic levels are higher than those reported in other tissues (Blaner and Olson, 1994). For example, we found that the choroid produces 40 times more retinoic acid than does chicken liver (liver produces approximately 750 femtomoles mg protein⁻¹ hr⁻¹; cf. Table I). After incubating the choroid with labeled retinol, we found that the medium contained levels of

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<th>Table I</th>
<th>Conversion of retinol to all-trans-retinoic acid in chick eye</th>
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<tr>
<td>Retina + RPE</td>
<td>1330 ± 300</td>
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<tr>
<td>Choroid</td>
<td>10600 ± 3000</td>
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<td>Sclera</td>
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RPE, retinal pigment epithelium; ND, not detected (detection limit of the assay was 200 femtomoles mg protein⁻¹ hr⁻¹ and 25 femtomoles punch⁻¹ hr⁻¹). Data presented (mean ± s.d.) are from separate experiments (n = 4) for two columns.
choroid (for 1 day at 6–7 days of age), which increase the rate of between the two groups. (b) Negative lenses (for 3 days) after 11 days, which decreases the di user (after 11 days of wear), which reveals the eye’s myopia and results in slowed ocular elongation and rapid recovery from the myopia, causes the synthesis of all-trans-retinoic acid to increase to a level four–five times that of controls [Fig. 2(A)].

The same bi-directional modulation of choroidal retinoic acid synthesis occurs after spectacle lenses are worn for a day. Negative lenses, which shift the image plane further back in the eye and increase the rate of ocular elongation as the animal compensates for this shift, cause decreased synthesis of all-trans-retinoic acid [Fig. 2(B)]; positive lenses, which shift the image forward in the myopic direction and decrease the rate of ocular elongation, result in increased synthesis of all-trans-retinoic acid [Fig. 2(C)], as does the myopic defocus resulting from removal of a diffuser [Fig. 2(A)].

The time course of the synthetic changes differs: removal of diffusers causes the rate to nearly double in 6 hr, a significant increase [unpaired t-test, P < 0.01; Fig 3(a)]; putting on diffusers causes a slow decline to about half in 12 hr [unpaired t-test, P < 0.05; Fig. 3(b)]. In both conditions, slightly more retinoic acid was found in the medium than in the tissue, as was the case in Fig. 1. The two visual conditions that cause increased retinoic acid secretion also cause dramatic thickening of the choroid (Wallman et al., 1995), with an approximately similar time course (unpublished data).

Thus choroidal all-trans-retinoic acid synthesis is inversely related to the rate of ocular elongation, rather than being associated with any particular visual condition. It is high under conditions leading to decreased ocular elongation (either positive lenses or recovery from deprivation myopia), and low under conditions leading to increased elongation (either negative lenses or form-deprivation).
Choroidal Retinoic Acid in Medium is Found Associated with Specific Proteins

The movement of retinoids through the body and cells is facilitated by specific retinoid binding proteins (Sporn, Roberts and Goodman, 1994). To determine if the retinoic acid released by the choroid is associated with a specific protein in culture medium, we used UV photolysis (Bernstein et al., 1995), to link tritiated retinoic acid released by choroidal tissue to associated proteins. This linking resulted in the labeling of proteins with a molecular weight range of approximately 28–32 kDa (Fig. 4). Incubation with an excess of all-trans-retinoic acid prior to UV-cross-linking results in a decrease in labeling; incubation with a mixture of all-trans- and 9-cis-retinoic acid eliminated the labeling. In contrast, pre-incubation with an excess of 13-cis-retinoic acid did not displace the labeling, nor did incubation with an isomeric mixture of either retinol or retinaldehyde (data not shown). These results indicate that the binding of retinoic acid to these proteins is highly specific.

Determination of the in vivo Levels of Retinoic Acid in Scleral Tissue

To assess the amount of retinoic acid found in the sclera, we did HPLC on pooled samples from five eyes. To confirm that the peak measured [Fig. 5(B)] is all-trans-retinoic acid we (a) showed that it eluted at the same time as known all-trans-retinoic acid from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and (b) compared the UV absorption spectrum of this peak to that of known all-trans-retinoic acid and found that they were identical (data not shown). The concentration of retinoic acid in normal chick sclera is approximately $5 \times 10^{-10} \text{M}$. This value was determined by calculating the amount of retinoic acid from the area under the retinoic acid peak in the HPLC profile and calculating the volume of the punch of scleral tissue by assuming an average thickness of 100 µm.

Retinoic Acid Alters Scleral Proteoglycan Formation

As the size of the eye is determined by the sclera, and scleral proteoglycan synthesis has been shown to be correlated with changes in the rate of ocular elongation (Rada, Thoft and Hassell, 1991), we measured the effect of all-trans-retinoic acid on the synthesis of scleral proteoglycans in vitro. This treatment caused a dramatic dose-dependent decrease in the incorporation of sulfate precursors into proteo-
approximately 40% of scleral proteoglycan synthesis would be inhibited. If we assume that the rate of choroidal retinoic acid released in vivo is similar to the in vitro rate of 1.6–2.4 pmole cm⁻² hr⁻¹ (Table I), that the normal choroid is 250 µm thick (Nickla, Wildsoet and Wallman, 1998), that the sclera is 100 µm thick, that retinoic acid diffuses freely in the choroid and sclera and that very little of the retinoic acid released into the choroid is removed by the blood, then the EC₅₀ of retinoic acid would be attained within 5 min of synthetic activity.

As choroidal cells release retinoic acid into culture medium and retinoic acid alters scleral proteoglycan formation, we measured the transfer to the sclera of retinoic acid released into the medium by the choroid in co-culture. Culturing choroid and sclera together with tritiated retinol for 2 hr and then isolating a nucleus-enriched fraction (Feeney-Burns and Berman, 1982) of the sclera, we found that this fraction was highly enriched in all-trans-retinoic acid. Although the scleral punch represents less than 1% of the total volume in culture (scleral volume was 3.8 µl of a total volume of 500 µl), it accumulated more than 50% of the all-trans-retinoic acid released by the choroidal tissue into the medium (290 ± 50 femtomoles; Table I and Fig. 1).

4. Discussion

We have presented a set of results which link visual inputs to changes in choroidal synthesis of retinoic acid, and also link choroidal retinoic acid to the modulation of scleral extracellular matrix proteoglycans. With respect to the first of these links, we found that the choroid synthesizes large amounts of retinoic acid, even more than the retina. Over half of the retinoic acid synthesized by the choroid (but only 10% of that synthesized by the retina) is found in the medium, suggesting either that it is actively secreted, or that it passively diffuses out of the cells that synthesize it, but is excluded from some compartments of the choroid. In either case, some characteristic particular to the choroid favors the release of retinoic acid into the medium. The changes in choroidal retinoic acid synthesis measured in vitro after lens-wear appear to reflect changes that occur in vivo because we have found comparable changes in the levels of endogenous retinoic acid in the choroid when birds wear positive or negative lenses for three days (Mertz et al., 1999).

The modulation of choroidal retinoic acid by specifically those visual conditions that affect ocular elongation is compatible with retinoic acid being part of a signal cascade from retina to sclera, modulating ocular elongation and thereby influencing refractive error. We find that two rather different visual manipulations, wearing a diffuser and wearing a negative lens, both cause increased ocular elongation and both cause decreased synthesis of choroidal glycosaminoglycans (Fig. 6). The EC₅₀ for all-trans-retinoic acid is 8 × 10⁻⁸ M. At the endogenous levels of retinoic acid in untreated scleras from normal eyes (5.5 × 10⁻⁸ M),
retinoic acid. Furthermore, two other rather different manipulations, wearing positive lenses and the removal of a previously worn diffuser, both cause decreased ocular elongation and both cause increased synthesis of choroidal retinoic acid.

To clarify whether these associations signify that retinoic acid is part of a signal cascade leading to altered eye growth or are epiphenomena of changes in the level of neuronal activity in the retina, we measured retinoic acid synthesis in two visual conditions which have large effects on ocular elongation, but would be expected to have small effects on the average level of retinal activity. First, we found that brief daily periods of stroboscopic light at dawn and dusk, which are known to inhibit the ocular elongation resulting from wearing diffusers (Nickla, 1996), also prevent the decline in choroidal retinoic acid synthesis usually found in eyes wearing diffusers unpub. data. Second, we found that very brief episodes of positive or negative lens-wear (2 min hr⁻¹ of lens-wear for 3 days, darkness the rest of the time) produce significant compensatory changes in the rate of ocular elongation and also produce substantial and significant changes in the abundance of retinoic acid in the choroid plus sclera. Positive lenses decreased the rate of ocular elongation and increased the abundance of retinoic acid; negative lenses did the opposite (Winer, Mertz and Wallman, unpublished data). These results further support the tight association between the visual conditions that affect choroidal retinoic acid and those that affect ocular elongation, suggesting that the association may be causal, rather than fortuitous.

In the retina, as well, visual conditions influence the levels of retinoic acid. Form-deprivation causes a 21% increase in the retinoic acid content (Seko, Shimizu and Tokoro, 1998). This change is much less than the change in the level of synthesis in the choroid and is in the opposite direction. We have preliminary evidence that the wearing of positive or negative spectacle lenses also modulates retinoic acid levels in the retina, again in the opposite direction from the changes in choroidal retinoic acid levels (Mertz et al., 1999). Thus, retinal retinoic acid might also be a participant in the same signaling cascade as the choroidal retinoic acid. If this is so, it is unlikely that retinal retinoic acid directly inhibits choroidal retinoic acid synthesis because in our tissue culture system exogenous retinoic acid does not inhibit choroidal retinoic acid synthesis.

We propose that the choroid synthesizes retinoic acid, which is transported to the sclera and modulates its growth. This role for the choroid is not an obvious one, first, because blood vessels predominately in the choroid, and blood vessels have not been shown to be a source of retinoic acid elsewhere in the body, and second, because the abundant blood flow in the choroid and the fenestrated capillaries of the choriocapillaris might be expected to carry away any small molecules produced there. We suspect that the retinoic acid is produced not by the blood vessels but by the lamina fusca at the posterior margin of the choroid, which places the source closer to the sclera than to the choriocapillaris at the anterior side of the choroid. Immunohistochemistry shows that the choroid contains two proteins associated with retinoid metabolism: cellular retinaldehyde binding protein, concentrated in the posterior choroid, and retinaldehyde dehydrogenase, located throughout the choroid with strong staining in the outer choroid (Fischer et al., 2000). Furthermore, choroidal retinoic acid being bound to a protein might reduce the diffusional losses.

Our results suggest that the transport of retinoic acid to the sclera is facilitated by scleral tissue having a special affinity for retinoic acid. When a piece of sclera amounting to one percent of the volume of the culture was cultured together with a piece of choroid, the nucleus-enriched fraction of the sclera acquired in 2 hr about half of the retinoic acid synthesized. Although it is difficult to extrapolate from these in vitro results to estimate the concentrating ability of the sclera in vivo, it seems plausible that if retinoic acid is produced in the outer choroid, much of it would end up in the nuclei of scleral cells.

As extracellular retinoic acid is always found associated with retinoid binding proteins, the fact that the retinoic acid synthesized by the choroid is bound to a protein is also compatible with its transport to the sclera. Although we know little about this protein, it may be a novel retinoid-binding protein. Of the previously described extracellular retinoic acid binding proteins, albumin and interphotoreceptor retinoid binding protein have higher molecular weight and bind all isomers of retinoic acid, and lipocalin-type prostaglandin D synthase, which binds retinoic acid and is released from RPE cells into the interphotoreceptor space, is slightly smaller (21–26 vs 28–32 kDa) and binds retinaldehyde 13-cis-retinoic acid with a similar kD as that for retinoic acid (Tanaka et al., 1997). The choroidal retinoic acid binding protein may, however, be similar or identical to the 28 kD protein found in the RPE by Bernstein et al. (1995).

Finally, we have shown that retinoic acid in vitro strongly inhibits the synthesis of GAGs, which are major constituents of the proteoglycans in the extracellular matrix. Others have shown that retinoic acid in tissue culture inhibits proliferation of scleral fibroblasts and chondrocytes, the two major cell types in the avian sclera (Seko, Shimokawa and Tokoro, 1996). As the concentration of retinoic acid found in the sclera of normal eyes is close to the center of the dose-response curve (50% inhibition), retinoic acid might modulate proteoglycan synthesis bidirectionally. Thus, the levels of retinoic acid in eyes wearing negative lenses might be low enough that the decreased inhibition could account for part or all of the increased proteoglycan synthesis observed with negative lenses (Marzani and Wallman, 1997; Nickla et al.,
1998); in addition, the increase in retinoid acid levels with positive lenses could account for part or all of the growth inhibition observed with these lenses. Therefore, sclera, which does not synthesize retinoid acid, has the receptors for retinoid acid (both nuclear retinoid acid receptors and retinoid receptors and retinoid x receptors) (Fischer et al., 2000). These might be used to modulate proteoglycan synthesis and cell division. Our results imply that the source of this retinoid acid could be the adjacent choroid.

If choroidal retinoid acid is a major intermediary between altered visual conditions and subsequent changes in ocular elongation, what retinal factors might regulate its synthesis? As mentioned above, there are retinal signals, such as dopamine, acetylcholine, glucagon and retinoid acid, with links to myopia. Perhaps a retinal signal such as one of these induces the retinal pigment epithelium to secrete a signal that modulates choroidal retinoid acid secretion, which in turn modulates scleral growth and thereby ocular elongation. If this is so, it is plausible that the myopia that children commonly develop during the school years may be associated with decreased choroidal secretion of retinoid acid and that pharmacological stimulation of choroidal retinoid acid synthesis might ameliorate myopic progression.

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