Prostaglandin-Induced Iris Color Darkening

An Experimental Model

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Objectives: To determine the role of sympathetic innervation and the effect of topical prostaglandin therapy on iris color in pigmented rabbits.

Methods: Twelve Dutch-belted rabbits underwent unilateral superior cervical ganglionectomy (SCGx) at age 1 to 3 months. A second group of 11 rabbits underwent bilateral SCGx at age 1 month and were treated once or twice daily for 6 to 9 months with 1 drop (about 20 µL) of latanoprost, 0.005%, to one eye and its vehicle to the contralateral eye. Standardized color photographs of the iris of each eye were taken at 1- to 2-month intervals for 6 to 10 months and evaluated by 4 to 6 observers in a masked fashion.

Results: At 8 to 10 months after unilateral SCGx, 11 of 12 rabbits showed definite heterochromia, with the lighter-colored iris on the SCGx side. Of the 11 rabbits that underwent bilateral SCGx and unilateral latanoprost treatment, 9 showed heterochromia at 6 to 9 months, with the darker-colored iris on the latanoprost-treated side.

Conclusions: These results demonstrate that sympathetic innervation is required for age-related, physiologic darkening of iris color in rabbits, that prostaglandins may compensate for sympathetic denervation to produce darkening in SCGx eyes, and that this model may be useful to study prostaglandin-induced iris color change.


Recent reports demonstrate that with long-term latanoprost treatment, some patients have darkening of iris color1-6 and eyelashes.4,7,8 In those patients exhibiting iris color changes, the baseline iris color was described as green-brown, yellow-brown, or blue-gray-brown, with a darker color around the sphincter than in the periphery. Latanoprost treatment caused darkening of the lighter-colored peripheral iris, resulting in a more uniformly brown color. Iris color darkening was not observed in any patient who had a uniformly blue or brown iris color at baseline.1-6

The precise mechanism of this iris color darkening is unknown. Iris color darkening occurs in cynomolgus monkey eyes treated with relatively high doses of latanoprost, a prostaglandin (PG) F2α analog, or with any of the naturally occurring PGS.9 Arachidonic acid products that are known to stimulate growth and possibly melanogenesis in normal human epidermal melanocytes in tissue culture include PGE2, PGD2, leukotriene B4, leukotriene C4, leukotriene E4, thromboxane B2, and 12-hydroxyeicosatetraenoic acid.10 On the other hand, PGE1, PGF2α, and 6-keto-PGF1α do not exhibit a stimulatory effect.10 Iris color is determined by the amount of melanin (melanosomes) within iris stromal melanocytes, not by the number of melanocytes.11-13 A uniformly blue iris has as many stromal melanocytes as a dark brown iris. Prolonged PG treatment has been shown to increase the eumelanin content of the melanocytes, but not the number of melanocytes in monkey irides.9

Neurohumoral input, including sympathetic innervation, is known to influence iris color.14,15 Sympathetic innervation plays a role in the development of normal pigmentation of the iris at birth.17 A hallmark of unilateral congenital Horner syndrome is iris heterochromia, with a lighter-colored iris on the side of the sympathetic deficiency. Less well known is the observation that acquired Horner syndrome also can produce a lightening of iris color,16,18 suggesting that sympathetic innervation may be required to maintain normal iridial pigmentation, even in adults. Unilateral sympathectomy in rabbits, regardless of age, is known to produce het-
MATERIALS AND METHODS

Twelve Dutch-belted female rabbits underwent unilateral superior cervical ganglionectomy (SCGx) at age 1 to 3 months (group 1). They were anesthetized with an injection of ketamine hydrochloride (20 mg/kg), xylazine hydrochloride (8 mg/kg), and acepromazine maleate (1 mg/kg), and SCGx was performed with the aid of a dissecting microscope. The occurrence of miosis postoperatively was initial evidence that sympathetic denervation had been achieved. Six months after surgery, the adequacy of the SCGx was tested pharmacologically with topical 10% cocaine hydrochloride; 1% hydroxyamphetamine hydrobromide; and 0.25% phenylephrine hydrochloride.

Color photographs of the iris of each eye were taken at 1- to 2-month intervals for 10 months. Four or 5 photographs of each eye were taken in a darkened room using a fundus camera. Flash intensity, magnification, F-stop, exposure time, and film type were consistent for all photographs. Slides of the right and left eyes of each rabbit were projected side-by-side in a darkened room. Four to 6 masked observers were asked to choose the darker of the 2 irides by indicating ++ for very certain, + for certain, and – for undecided. For each rabbit, 4 or 5 pairs of slides were judged at each time interval. The ratings from each rabbit at all time intervals were pooled and the degree of certainty of heterochromia between contralateral eyes of rabbits among the observers was rated as follows: strong (very certain was the unanimous opinion of all observers); moderate (1 or 2 observers might have disagreed with the others, or there might have been a slight inconsistency at some intervals during the course of treatment); mild (although there was a trend toward heterochromia, considerable variability existed); or no consistently distinguishable difference between eyes.

A separate group of 12 rabbits underwent bilateral SCGx at age 1 month (group 2). One rabbit died on the day following surgery. Four days after surgery, 1 drop (about 20 µL) of 0.005% latanoprost, 10% cocaine hydrochloride; 1% hydroxyamphetamine hydrobromide; and phenylephrine hydrochloride. A separate group of 12 rabbits underwent bilateral SCGx at age 1 month (group 2). One rabbit died on the day following surgery. Four days after surgery, 1 drop (about 20 µL) of 0.005% latanoprost, 10% cocaine hydrochloride; 1% hydroxyamphetamine hydrobromide; and phenylephrine hydrochloride. One rabbit died on the day following surgery. Four days after surgery, 1 drop (about 20 µL) of 0.005% latanoprost, 10% cocaine hydrochloride; 1% hydroxyamphetamine hydrobromide; and phenylephrine hydrochloride.

The heterochromia produced after unilateral SCGx in these pigmented rabbits (group 1) confirmed the results of previous studies and is consistent with the heterochromia that is observed clinically in patients with Horner syndrome, especially when it occurs congenitally. Iris color undergoes changes during aging and appears to be influenced by neurohumoral factors. The lightening of iris color in some patients who develop acquired Horner syndrome as adults suggests that sympathetic tone contributes to maintenance of iris color in adulthood.

An interaction between PGs, adrenergic drugs, and the sympathetic nervous system has been reported previously. Cyclooxygenase inhibitors suppress the ocular hypotensive effects of adrenergic agents and suppress the reduction in intraocular pressure normally seen following acute superior cervical ganglionectomy. In toxicology studies, none of the hundreds of pigmented rabbits with intact sympathetic innervation of the iris that were treated with high doses of latanoprost for more than a year demonstrated darkening of iris color (Johan Stjernschantz, MD, PhD, Pharmacia & Upjohn, Kalamazoo, Mich, unpublished data, 1994). This study examines the relationship of sympathetic denervation and latanoprost treatment to the development of iris hyperpigmentation in a rabbit model.

RESULTS

At 8 to 10 months after unilateral SCGx, 11 of the 12 rabbits in group 1 showed definite heterochromia, with the lighter-colored iris on the SCGx side, as determined unanimously by the masked observers (Figure). The heterochromia was observed as soon as 2 weeks after SCGx in the youngest rabbits.

Of the 11 rabbits in group 2 that underwent bilateral SCGx and unilateral latanoprost treatment, 10 showed some degree of heterochromia after 6 months of treatment: 5 rabbits, strong degree of certainty; 3 rabbits, moderate; and 2 rabbits, mild (Table). The darker eye was found to be the latanoprost-treated eye in 9 of the 10 rabbits (Figure). The only rabbit that received latanoprost in the eye thought to be lighter in color received a heterochromia rating with a mild degree of certainty. The 1 rabbit that did not show heterochromia was the only 1 of the 11 with an incomplete SCGx, as demonstrated by pupillary testing with cocaine hydrochloride, hydroxyamphetamine hydrobromide, and phenylephrine hydrochloride.

COMMENT

The heterochromia produced after unilateral SCGx in these pigmented rabbits (group 1) confirmed the results of previous studies and is consistent with the heterochromia that is observed clinically in patients with Horner syndrome, especially when it occurs congenitally. Iris color undergoes changes during aging and appears to be influenced by neurohumoral factors. The lightening of iris color in some patients who develop acquired Horner syndrome as adults suggests that sympathetic tone contributes to maintenance of iris color in adulthood.

The interaction between PGs, the sympathetic nervous system, and adrenergic agonists has been previously delineated. In clinical and/or animal studies, cyclooxygenase inhibitors prevented the ocular hypotensive effects of adrenergic agents (reviewed by Camras and Podos, 1989) and inhibited the intraocular pressure decrease after acute superior cervical ganglionectomy. With intact sympathetic innervation of the iris, high doses of latanoprost for more than a year do not result in darkening of iris color in pigmented rabbits (Johan Stjernschantz, MD, PhD, Pharmacia & Upjohn, Kalamazoo, Mich, unpublished data, 1994). However, the results
Irides of 2 Dutch-belted rabbits. Top, Eyes of a rabbit 6 months after surgical removal of the right unilateral superior cervical ganglion. The right eye (A) was judged to be lighter in color than the left eye (B) by 6 masked observers. Bottom, Eyes of a rabbit 6 months after the surgical removal of both superior cervical ganglia. The left eye (D) had been treated with vehicle and the right eye (C) with 0.005% latanoprost, once or twice daily for 6 months after the surgery. The right eye was judged to be darker than the left eye by 6 masked observers.

<table>
<thead>
<tr>
<th>Effects of Unilateral Latanoprost Treatment on Iris Color in 11 Rabbits Following Bilateral Superior Cervical Ganglionectomy (Group 2)*</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No obvious heterochromia</td>
<td>1/11 (9)</td>
</tr>
<tr>
<td>Heterochromia†</td>
<td>10/11 (91)</td>
</tr>
<tr>
<td>Strong degree of certainty</td>
<td>5/10 (50)</td>
</tr>
<tr>
<td>Moderate degree of certainty</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>Mild degree of certainty</td>
<td>2/10 (20)</td>
</tr>
<tr>
<td>Darker eye treated with latanoprost</td>
<td>9/10 (90)</td>
</tr>
<tr>
<td>Darker eye treated with vehicle (mild certainty)</td>
<td>1/10 (10)</td>
</tr>
</tbody>
</table>

*0.005% Latanoprost was applied once or twice daily to one eye and the vehicle was applied to the contralateral eye in a double-masked, randomized fashion for 6 to 9 months. Photographs were evaluated by 4 to 6 observers in a masked fashion.
†Strong indicates that the opinion of all observers was unanimous; moderate, 1 or 2 observers might have disagreed with the others, or there might have been a slight inconsistency at some intervals during the course of treatment; and mild, in the opinion of the observer, although there was a trend toward heterochromia, considerable variability existed.

of the present study suggest that PGs may act in the absence of sympathetic tone by stimulating melanogenesis to darken the color of irides that are lighter following sympathectomy.

Iris stromal melanocytes in the iris periphery are preferentially innervated by the sympathetic nerve endings, whereas those around the sphincter of the iris tend to be cholinergically innervated.10 Iris freckles/nevi are not sympathetically innervated and therefore do not fade in Horner syndrome.24 If PGs were substituting for deficient sympathetic innervation, the peripheral rather than more central iris stroma would be expected to darken, which is consistent with the clinical observations.1-6 Furthermore, latanoprost-induced darkening of neviformations would not be expected and in fact does not occur. Among the hundreds of eyes treated with latanoprost that have been carefully evaluated photographically in clinical studies, none of the iris freckles/nevi have demonstrated any change. Even large irides observed at baseline do not change during the course of 1 year of treatment with latanoprost.1-6

Darkening of the iris after topical latanoprost treatment does not appear to be due to proliferation of melanocytes.9 In our experimental rabbit model, histopathologic studies are being performed to determine whether the iris color darkening is due to proliferation of melanocytes or an increase in melanin synthesis within the melanocytes. The model of sympathetically denervated rabbit eyes may be useful to further study the mechanism of PG-induced iris color change.

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REFERENCES


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