**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.


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**Recent reports demonstrate** that with long-term latanoprost treatment, some patients have darkening of iris color and eyelashes. 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.

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. Arachidonic acid products that are known to stimulate growth and possibly melanogenesis in human epidermal melanocytes in tissue culture include PGE₂, PGD₂, leukotriene B₄, leukotriene C₄, leukotriene E₄, thromboxane B₂, and 12-hydroxyeicosatetraenoic acid. On the other hand, PGE₁, PGF₂α, and 6-keto-PGF₁α do not exhibit a stimulatory effect. Iris color is determined by the amount of melanin (melanosomes) within iris stromal melanocytes, not by the number of melanocytes. 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.

Neurohumoral input, including sympathetic innervation, is known to influence iris color. Sympathetic innervation plays a role in the development of normal pigmentation of the iris at birth. 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, 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-
the sympathetic nervous system has been reported prethectomy side.14-16 Also, topical application of an a-adrenergic blocker results in iris heterochromia in young rabbits.19

An interaction between PGs, adrenergic drugs, and the sympathetic nervous system has been reported prevously. Cyclooxygenase inhibitors suppress the ocular hypotensive effects of adrenergic agents20 and suppress the reduction in intraocular pressure normally seen following acute superior cervical ganglionectomy.21 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 studies14,15,22 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 aging17,18 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.16,18

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,20 1989) and inhibited the intraocular pressure decrease after acute superior cervical ganglionectomy.21 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, unpublished data, 1994). However, the results...
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. 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. Furthermore, latanoprost-induced darkening of nevi/freckles 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 iris nevi observed at baseline do not change during the course of 1 year of treatment with latanoprost.

Darkening of the iris after topical latanoprost treatment does not appear to be due to proliferation of melanocytes. 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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