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.

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 was applied to each eye. Two rabbits were treated with high doses of latanoprost for more than a year do not result in iris hyperpigmentation in young rabbits.19

An interaction between PGs, adrenergic drugs, and the sympathetic nervous system has been reported previously. Cyclooxygenase inhibitors prevent 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, Kalamazo, Mich, unpublished data, 1994). This study examines the relationship of sympathetic denervation and latency 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 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 studies14-16,22 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 aging17,23 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, Kalamazo, 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. Iris freckles/nevi are not sympathetically innervated and therefore do not fade in Horner syndrome. 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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