
Adrenergic influence on iris pigmentation in newborn pigmented rabbits was studied. Selective adrenergic antagonists were used topically to determine whether they could inhibit iris pigmentation. Unilateral, topical administration of an alpha-adrenergic antagonist (thymoxamine hydrochloride ½%) was associated with iris hypochromia, but identical treatment with a beta-adrenergic antagonist (timolol ½%) was not associated with iris hypochromia. Adrenergic influence on iris stromal melanogenesis appears to be mediated by alpha-adrenergic receptors. (Invest Ophthalmol Vis Sci 23:528-530, 1982.)

More than 60 years ago, Bistis suggested that some cases of heterochromia iridis in humans were caused by a congenital, or at least early-onset, paralysis of the sympathetic innervation of the eye. Calhoun confirmed these clinical observations and he demonstrated heterochromia iridis in newborn rabbits subjected to interruption of the cervical sympathetics. In clinical practice, a Homer's syndrome of congenital or early-onset is now a well-established cause of heterochromia iridis. The heterochromia is due to hypopigmentation of the affected iris. Appreciation of iris hypochromia in a newly recognized Homer's syndrome points to a long-standing lesion of benign etiology. It is therefore a very helpful clinical finding.

The manner in which sympathetic innervation of the iris affects iris stromal melanocytes is open to question. An early theory explaining the association of sympathetic paralysis and hypopigmentation of the iris was that sympathetic paralysis resulted in a trophic disturbance mediated by the blood vessels of the iris. According to published reports, iris stromal melanocytes deprived of some nutritive factor failed to populate the iris stroma and/or failed to produce pigment. More recently, attention has focused on a more direct influence of adrenergic innervation on stromal melanocytes. Ultrastructural studies by Latties and by Ringvold suggested close contact between sympathetic axons and iris melanocytes in rabbits. Moreover, experimental interruption of cervical sympathetics causes a reduction in iris tyrosinase activity. Somehow sympathetic innervation of the eye stimulates melanogenesis in iris stromal melanocytes.

Although the exact mechanism of sympathetic influence on iris stromal melanogenesis remains incompletely understood, we designed a simple in vivo experiment to determine whether this adrenergic influence was mediated by alpha-adrenergic or beta-adrenergic receptors.

Materials and methods. We used selective adrenergic antagonists to identify whether iris pigmentation was under alpha-adrenergic or beta-adrenergic control. Thymoxamine hydrochloride is a selective alpha-adrenergic blocking agent. Timolol is a selective beta-adrenergic blocking agent. Newborn pigmented rabbits, of mixed Checkered and Flemish origin, were divided into three groups after inspection revealed no evidence at the time of lid separation of preexisting heterochromia. Rabbits with darkly pigmented irides were excluded from the study. Group I received thymoxamine hydrochloride ½% drops (prepared according to published reports) to the right eye at 8 A.M., 12 noon, and 4 P.M., and group II received timolol ½% (commercial product) drops to the right eye on the same schedule. Group III received no drops. The trial was initiated as soon as the lids could be separated and it continued for 12 weeks, at which time photographs of the irides were obtained prior to sacrifice of the animals. Light exposure was kept constant for the photographs. The rabbits were raised on a 12 hr daylight cycle. At no time did the eyes appear inflamed, but topical instillation of thymoxamine hydrochloride was associated with transient ocular irritation and miosis.

The photographs were judged in a double-masked fashion. Twenty-five observers were asked to decide whether each pair of eyes (right and left...
Figs. 1 to 3. Three animals treated with thymoxamine hydrochloride ½% topically to the right eye for 12 weeks after birth. Top, Right eye; bottom, left eye. These animals were judged by all of 25 observers to have hypochromia of the treated eye. (Animals 4 and 5 were not uniformly judged to have hypochromia of the treated eye; see text.)

Table I. Results of masked comparison of iris pigmentation in treated vs. untreated eyes of 10 rabbits judged by 25 observers

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Right eye hypochromic</th>
<th>Left eye hypochromic</th>
<th>Isochromic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (thymoxamine, right eye)</td>
<td>1</td>
<td>25/25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25/25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25/25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3/25</td>
<td>5/25</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>21/25</td>
<td>17/25</td>
</tr>
<tr>
<td>Group II (timolol, right eye)</td>
<td>1</td>
<td>25/25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25/25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25/25</td>
<td></td>
</tr>
<tr>
<td>Group III (no drops)</td>
<td>1</td>
<td>25/25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25/25</td>
<td></td>
</tr>
</tbody>
</table>

not identified) were equally pigmented or not. If not, they were asked which eye was more darkly pigmented. They were instructed to judge the iris stroma for pigment density and not just overall appearance. The photographs were then uncoded and the results tabulated. Histopathologic examination of the treated irides did not reveal any evidence of inflammation.

Results. In Group II (timolol treated) and group III (no treatment) there was no heterochromia appreciated by any of the observers (Table I). In group I (thymoxamine treated) all 25 observers felt that three of the five rabbits (animals 1, 2, and 3) demonstrated heterochromia (Figs. 1 to 3) and that the thymoxamine-treated eye was hypochromic. In the other two rabbits of group I, responses were variable, but most observers judged the treated eye to be hypochromic or isochromic.

Discussion. The study design of this experiment has some limitations. First, the treatment regimen did not inhibit melanogenesis completely because treated irides as well as untreated irides progressively darkened. This is to be expected, since the treatment did not provide for around-the-clock drop instillation. Timolol, for example, has been shown to inhibit beta-adrenergic stimulation of cyclic AMP in iris–ciliary body for only 3 hr after topical instillation. Second, these drops are systemically absorbed so that any potential differences between the two eyes would probably be minimized. Third, although the thymoxamine-treated eyes did not appear clinically or pathologi-
cally inflamed, it is remotely possible that a low-
grade iritis could have accounted for the hetero-
chromic iridis. Iritis has not been reported to be a side
effect of thymoxamine, but there is definitely
some transient ocular discomfort in animals and
humans treated topically with thymoxamine hy-
drochloride ½%. Our findings suggest that sympathetic influence
on uveal melanocytes is mediated by alpha-
adrenergic receptors. It is well known that the
dilator pupillae is also under alpha-adrenergic con-
trol. Since thymoxamine induces a reversible
miosis but timolol has no effect on pupil size, it is
remotely possible that miosis alone could some-
how have inhibited stromal melanogenesis. This
seems unlikely, since the miosis we observed was
temporary and mild.

These findings may have some clinical rele-
ance. Thymoxamine hydrochloride is currently
being evaluated clinically for use in a variety of
diseases. Our data predict that its use in infants
could be associated with heterochromia as a side
effect. Conversely, our data would predict that
timolol in infants with congenital glaucoma would
not cause heterochromia.

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melanocytes, heterochromia, hypochromia

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Lectin-mediated attachment of liposomes to
cornea: influence on transcorneal drug
flux. HELENE E. SCHAFFER, JULIANNA M.
BREITFELLER, AND DAVID L. KROHN.

A method to enhance retention of drug-bearing liposomes
at the corneal surface under conditions of tear flow was
investigated. Mixed brain gangliosides were incorporated
into the membranes of phosphatidyl choline liposomes to
provide receptor sites for wheat germ agglutinin, a plant
lectin that binds strongly to both human and rabbit
corneal epithelium. Ganglioside-containing liposomes
showed a 2.5-fold increase in their binding to rabbit cor-
nea in vitro when corneas were pretreated with wheat
germ agglutinin (500 μg/ml), suggesting that the lectin
mediates specific binding of these liposomes to the cor-
neal surface. In addition, under conditions of continuous
 tear flow (1 ml/h), ganglioside-containing liposomes
with entrapped carbachol significantly enhanced car-
bachol flux across isolated rabbit corneas pretreated with
wheat germ agglutinin 90 min after drug delivery. The
data support the potential use of liposomes as a vehicle
for topical drug flux enhancement. (Invest Ophthal-
mol Vis Sci 23:530-533, 1982.)

Liposomes employed as topical drug delivery
vehicles have been shown capable of enhancing
corneal drug flux in a static in vitro system. Relative affinities
for the corneal surface of liposomes differing in charge suggested that liposomes bind
to the cornea electrostatically. However, under physiologic conditions such as tear flow and
lid action, electrostatic binding may be insufficient
to preclude elution of the liposome-drug complex;
methods to enhance retention of the latter at the
corneal surface include modification of the lipos-
some membrane to allow attachment of suitable
ligands that have a strong affinity for the corneal
epithelium. As a first step in this direction, we
have incorporated mixed brain gangliosides to
serve as receptor for the plant lectin, wheat germ
agglutinin (WGA), into the membranes of
phosphatidyl choline (PC) liposomes. WGA ex-
hibits a strong affinity for both human and rabbit
corneal epithelium (unpublished data) and, as a
bivalent molecule, can mediate the binding of
ganglioside-containing liposomes (GCL) to the
corneal surface. In this report we present evi-

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