Effects of Nicotinic Antagonists on Ocular Growth and Experimental Myopia

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PURPOSE. To learn whether nicotinic cholinergic receptors modulate postnatal eye growth and influence the course of form-deprivation myopia.

METHODS. One-week-old White Leghorn chicks wore a unilateral goggle to induce form-deprivation myopia. Other chicks were never goggled. Nicotinic antagonist drugs were administered by intravitreal injection, usually daily or every other day to the goggled eye or to one eye of never-goggled chicks. After 1 week, the eyes were studied by refractometry, A-scan ultrasonography, and caliper measurements.

RESULTS. The relatively non-subtype-specific channel-blocking nicotinic antagonists chlorisondamine and mecamylamine each inhibited the development of form-deprivation myopia but with complex multiphasic dose responses. Chlorisondamine was the most effective. Mecamylamine, at the lowest tested doses, tended to stimulate the growth response and myopic refractive shift of goggle wearing. Methyllycconitine competitively inhibits nicotinic receptors containing the α7 and α8 subunits, which are highly expressed in chick retina. It showed a less dramatic but still significant inhibitory effect on myopia. The effects of dihydro-β-erythroidine, a competitive antagonist relatively selective for nicotinic receptors with α5 or α4 subunits and particularly for α5β2-containing receptors, were the weakest and inhibited primarily axial elongation. Chlirisondamine but not mecamylamine also affected nongoggled eyes, inhibiting growth and shifting refraction toward hyperopia, but chlorisondamine also induced degenerative changes to the retinal pigment epithelium (RPE).

CONCLUSIONS. Nicotinic receptors are involved in eye growth control. Nicotinic antagonists affect the development of form-deprivation myopia and perhaps the growth of nongoggled eyes. The differences in drug activity and multiphasic dose-response curves may reflect the complexity of nicotinic receptor subtypes associated with the eye and/or pharmacokinetic differences between the individual drugs. Although another tissue(s) cannot be completely excluded by these data, the site of action of these agents may be neural retina or RPE. (Invest Ophthalmol Vis Sci. 2001;42:557–565)

Visual input dominates the regulation of postnatal eye growth and the development of refractive errors. Eye growth appears largely controlled locally in the eye, likely through the retina; specific roles for other components of the nervous system, such as the brain or peripheral nervous system, remain unclear.1,2 As complex qualities of the visual image such as blur influence eye growth, it seems reasonable that neurons in the proximal retina might comprise elements of a local regulatory circuit. Indeed, much evidence implicates several classes of retinal amacrine cells in the pathway linking visual input and eye growth control. The data most strongly support a role for dopaminergic amacrine cells.2,3 Although evidence implicating other retinal neurons is either less fully developed or controversial, other subtypes of retinal amacrine cells that might influence refractive development include those containing vasoactive intestinal peptide,13,14 glucagon,15 nitric oxide,16 enkephalin,17 and the subject of the present report, acetylcholine.18

Cholinergic mechanisms, acting through muscarinic receptors, seem involved in eye growth control because the muscarinic antagonist atropine retards the development of myopia in chick,19 tree shrew,20 monkey,11,12 and humans.13 However, identifying either the specific cholinergic neurons responsible for the regulation of eye growth or the mechanism by which muscarinic antagonists inhibit myopia development has proved difficult.12,14,16

Further limiting our understanding of the role of cholinergic neurons in eye growth control is the sparsity of studies addressing nicotinic cholinergic mechanisms. Like all birds, the iris and ciliary muscles of the chick are striated and contract through activation of nicotinic receptors; in contrast, nonvascular muscles found in the chick choroid are believed to be smooth muscle and to contract through muscarinic receptors.17,18 Accordingly, both intravitreal and subconjunctival nicotine induce accommodation and miosis in chicks.19 Twice daily intravitreal injections of nicotine for 2 weeks in chicks induced about a 2 diopter myopic shift in refraction compared with contralateral noninjected eyes, but intravitreal saline injection did the same.19 Daily subconjunctival nicotine injections in chicks did cause a slight myopic refractive shift of 0.75 diopters compared with nontreated eyes, a response not seen for subconjunctival saline,19 but this degree of refractive shift in chicks may be of little biological significance because it approximates the focal depth of the chick eye.20 Nicotine’s high lipophilicity would permit rapid diffusion from the eye, and potential action at extracocular sites further limits mechanistic interpretation of these results. The corneal application in chicks of vecuronium bromide, a neuromuscular blocking agent and nicotinic antagonist, paralyzed accommodation but failed to influence the ocular elongation after spectacle-induced hyperopic defocus, arguing against an accommodative mechanism for myopia.21 Charged antagonists at the neuromuscular junction, of which 4-tubocurarine is a prototype, typically penetrate poorly into the central nervous system and bind to all nicotinic receptor subtypes with low affinity.22 Vecuronium bromide is similarly highly charged, and although diffusing readily to block the neuromuscular junctions of in-
traocular muscles, it may have more limited access to receptor sites in lipophilic tissues potentially involved in eye growth control, such as the neural retina. These results overall thus preclude any conclusion regarding nicotinic mechanisms in eye growth control.

Besides the nicotinic acetylcholine receptors at the neuromuscular junctions of the iris and ciliary muscles, the chick eye has well-characterized nicotinic receptor subtypes in both retina and ciliary ganglion. We studied antagonists with established profiles against neuronal nicotinic receptors and with lipophilic properties compatible with diffusion into neural tissues. We found evidence for a potential role, perhaps a central role, of nicotinic receptors in eye growth control.

**Materials and Methods**

One-day-old white leghorn chicks (Truslow Farms, Chestertown, MD) were reared in brooders on a 12-hour light-dark cycle with General Electric chroma 50 fluorescent lighting with irradiance of approximately 50 μW/cm² at chick eye level. The chicks received Purina Chick Chow food and water ad libitum.

Experiments started at 1 week of age. For some chicks, a unilateral translucent white plastic goggle was glued to the periorbital feathers with cyanoacrylate glue to induce form-deprivation myopia. Under aseptic conditions, the goggled eye received a 10-μl intravitreal injection of either drug or saline vehicle at that time. Other chicks were nongoggled but similarly received intravitreal injections of either drug or vehicle to one eye. Drug or vehicle was administered by intravitreal injection at approximately 4 hours into the light phase on a daily or alternate day regimen for most experimental groups. In each series, the experimental eye was alternated between left and right, and all contralateral eyes received injections of saline vehicle at the same time as injections to the experimental eye. Each cohort of drug-treated goggled chicks included a group of vehicle-treated goggled birds as contemporaneous controls. Chicks were anesthetized with inhalation ether for all goggle applications and drug injections.

After 1 week of treatment and at 2 weeks of age, the chicks were anesthetized with an intramuscular mixture of ketamine (20 mg/kg) and xylazine (5 mg/kg), and ocular refractometry and A-scan ultrasonography were performed as described. No intracocular injections were administered on the day of examination. While still under general anesthesia, the chicks were decapitated, and the axial and equatorial dimensions of enucleated eyes were measured with digital calipers. The coronal profile of the chick eye is elliptical, and the equatorial diameter is reported as the mean of the shortest and longest equatorial dimensions of the eye.

The following drugs were administered daily: dihydro-b-erythroidine hydrobromide (RBI/Sigma, Natick, MA), mecamylamine (RBI/Sigma), and methyllycaconitine citrate (RBI/Sigma). Because it is a long-acting nicotinic antagonist in mammalian brain, chlorisondamine diiodide (Toxicos Cookson, Ballwin, MO) was administered every other day by intravitreal injection.

To assess acute drug effects, other 2-week-old chicks (n = 5/group) received a single unilateral intravitreal injection of one of the nicotinic antagonists at doses chosen on the basis of drug effects on the growth of goggled eyes: 200 μg chlorisondamine, 50 and 1 μg mecamylamine, 5 μg methyllycaconitine, or 50 μg dihydro-b-erythroidine. Just before and at 2 and 24 hours after injection, both eyes were examined by refractometry and ultrasonography by the above methods. Because chicks in the eye growth studies did not receive drug on the day of measurements, the 24-hour examination point was selected specifically to identify a potential residual drug effect on the intraocular muscles at a time relevant to the eye growth measurements.

To identify potential histopathologic effects in other groups of monocularly deprived or never-goggled 1-week-old chicks, chlorisondamine (200 or 100 μg every other day; n = 5–6/group), mecamylamine (200 or 50 μg daily; n = 5–8/group), or saline vehicle (n = 3–5/group) was administered by intravitreal injection to the goggled eyes or to one of the open eyes of never-goggled chicks with vehicle to the contralateral eye, using the identical protocol as above. After 1 week of treatment, the above protocols provided refraction, ultrasound, and caliper measurements. The eyes were then immersion fixed in 3% glutaraldehyde/0.5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The posterior segments were either embedded in paraffin, cut at 5-μm thickness, and stained with hematoxylin and eosin, or they were embedded in historesin, cut at 3- or 5-μm thickness, and stained with 0.5% azure II/0.5% methylene blue in 1% borate.

Data are provided as mean ± SEM and were analyzed with SigmaStat (SPSS, Inc., Chicago, IL). Neither visual deprivation nor drug treatments to these eyes affected lens thickness, and these data are not reported for goggled chicks. A one-way analysis of variance (ANOVA), using the differences between visually deprived and contralateral eyes on goggled chicks, was performed to ascertain drug efficacy against experimental myopia. Because the ultrasound data on axial length after mecamylamine treatment to goggled eyes did not meet conditions of normality, these data were assessed with a Kruskal-Wallis one-way ANOVA on ranks on the differences between experimental and contralateral eyes. Data from different cohorts of chicks tested with the same drug, along with the respective vehicle-treated controls, were pooled for analysis (Fig. 1). Because the drug effects in the never-goggled chicks also were not normally distributed, drug-treated nongoggled eyes and vehicle-treated contralateral eyes were compared with a Friedman repeated-measures ANOVA on ranks. In series when the ANOVA identified a treatment effect, post hoc multiple pairwise comparisons of the treatment groups were made with the Tukey test, using a value of P < 0.05 for statistical significance. In assessing acute drug effects on ocular refractions and ultrasounds, the measurements before and after drug injection were compared with a Student's paired t test. The experiments conformed with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Results**

**Goggled Chicks**

As expected from previous studies, the different cohorts of vehicle-treated control chicks wearing a unilateral goggle developed ipsilateral myopia of about −7 to −12 diopters compared with the contralateral nongoggled eyes (Fig. 1). The axial lengths in the goggled eyes were increased by approximately 0.4 to 0.6 mm compared with the contralateral eyes. In general, the axial length difference between goggled and open eyes was greater as measured by ultrasound, which records to the inner limiting membrane, than that measured by calipers, which records to the outer scleral surface. Besides the greater variability of the caliper measurements, this disparity may at least partly be physiologic because both the choroid and retina of young chicks thins during goggle wear. The vitreous cavity of goggled eyes was enlarged in both the axial and equatorial dimensions, with the vitreous cavity elongation largely accounting for the increase in overall axial length of the eye. Goggle wearing alone induced no significant effect on anterior chamber depth in most cohorts of vehicle-treated chicks.

Two relatively nonselective nicotinic antagonists were tested, chlorisondamine and mecamylamine. Chlorisondamine reduced the myopic refractive error (Fig. 1; ANOVA: P < 0.001), inhibited the excessive axial elongation developing beneath a goggle (Fig. 2; ANOVA: ultrasound, P < 0.001; calipers, P = 0.008), and reduced the vitreous cavity expansion in both axial (Fig. 2; ANOVA: P < 0.001) and equatorial (Fig. 2; ANOVA: P = 0.001) dimensions. Chlorisondamine had no statistically significant effect on anterior chamber depth (data not shown). Post hoc pairwise comparisons by the Tukey test (Table 1) showed significant drug effects compared with the vehicle-treated controls for refraction, axial length, and
vitreous cavity depth measurements and for several other intragroup comparisons.

The effects of mecamylamine on goggled eyes were more complex, with a multiphasic response that differed between the low and high drug doses. It had a maximal anti-myopia effect at the intermediate dose and tended toward stimulating the growth and myopic refractive shift of goggled eyes at the lowest doses. Mecamylamine overall altered refraction of goggled eyes (Fig. 1; ANOVA: $P < 0.001$). Although all three higher drug doses reduced the induced myopia, only the 50-μg dose differed significantly from the controls by post hoc pairwise comparison testing (Table 1) and virtually eliminated the induced myopia. Although the refractions at the 1- and 10-μg doses were not individually different from the controls by post hoc pairwise comparisons, significant differences occurred between the 1-μg dose and each of the 50-, 100-, and 200-μg doses as well as between the 10- and 50-μg doses (Table 1). The anatomic effects of mecamylamine on goggled eyes tended to follow the refractive effects: larger eyes developing with doses that did not reduce the myopia and smaller eyes with doses that did (Fig. 2). For axial length, there was only a trend toward a drug effect by ultrasound (ANOVA: $P = 0.07$); no statistical effect on axial length by caliper measurements was apparent. Mecamylamine influenced the ultrasound measurements of vitreous cavity length (ANOVA: $P = 0.007$); post hoc pairwise comparison testing identified the 1-μg dose as different from the 50-μg dose but not from the controls (Table 1). Similarly, overall equatorial diameter of goggled eyes was influenced by mecamylamine (ANOVA: $P = 0.003$); post hoc pairwise comparison testing did not identify any individual doses that differed from the controls but showed that the 1- and 50-μg doses differed from each other and that the 10-μg dose differed from both the 50- and 200-μg doses (Table 1).

There also may have been an anterior chamber effect from mecamylamine (ANOVA: $P = 0.009$), but post hoc pairwise comparison testing did not identify any individual differences. In reviewing the data on anterior chamber depth, the vehicle-treated goggled eyes in the mecamylamine experiments had an anterior chamber depth slightly shallower than the contralateral nongoggled eyes (1.22 ± 0.04 mm in goggled eyes versus 1.34 ± 0.04 mm in nongoggled eyes). The differences in anterior chamber depth between goggled and contralateral eyes were similar for the low mecamylamine doses, but for the 100- and 200-μg doses, the anterior chamber depths in the drug-treated, goggled eyes relative to the contralateral controls were no longer reduced but instead were equal (data not shown).

Of the antagonists with some subtype selectivity, methyllycaconitine showed the greater efficacy of the two drugs and was similar to mecamylamine in that the strongest effects seemed to occur at the intermediate drug doses (Fig. 2). Methyllycaconitine affected the myopic refraction (ANOVA: $P = 0.04$), axial length (ANOVA: ultrasound, $P = 0.05$; calipers, $P = 0.002$), and equatorial expansion of the vitreous cavity (ANOVA: $P = 0.02$) in goggled eyes (Figs. 1 and 2). A trend toward an influence on vitreous cavity length did not reach significance with this drug (Fig. 2; ANOVA: $P = 0.09$). With post hoc pairwise multiple comparisons by the Tukey test, a significant difference from controls was identified for the inhibition of equatorial expansion beneath a goggle only at the 5-μg dose; additionally, the 5-μg dose reduced axial length by calipers compared with the 0.05-, 0.5-, and 50-μg doses (Table 1). There was no effect from methyllycaconitine on the anterior chamber depth of goggled eyes (data not shown).

Dihydro-β-erythroidine exhibited only a weak effect against experimental myopia (Figs. 1 and 2). The drug induced a significant reduction only in axial length as measured by calipers (ANOVA: $P = 0.02$), but no individual drug dose was identified by the post hoc pairwise multiple comparison testing. Otherwise, none of the differences in refraction, ultra-
sound measurements, or caliper measurements of the equatorial diameter reached statistical significance by ANOVA.

**Nongoggled Chicks**

Unilateral intravitreal administration of chlorisondamine reduced the axial growth of drug-treated eyes in never-goggled chicks (Fig. 3; ANOVA on ranks: ultrasound, $P = 0.03$; calipers, $P = 0.05$). The growth reduction was confined to the vitreous cavity (ANOVA on ranks: $P = 0.01$) and was reflected in a hyperopic shift in refraction (ANOVA on ranks: $P < 0.004$). The effect on equatorial expansion of the vitreous cavity did not reach statistical significance. Pairwise comparisons with the Tukey test identified the refractions of the eyes treated with 200 and 100 $\mu$g and the vitreous cavity depths of the eyes treated with 100 and 50 $\mu$g as different from each other (Table 1). An effect on lens thickness also was noted (ANOVA on ranks: $P < 0.01$), comprising an increase of approximately 0.1 mm in both eyes in the 10-$\mu$g group compared with chicks receiving the 50-, 100-, or 200-$\mu$g doses as well as other chicks who received saline injections to both eyes; no pairwise comparisons of the lenses were identified as significant by the Tukey test, however.

In contrast to the chlorisondamine effects on open eyes, daily intravitreal injections of mecamylamine (data not shown) had no influence on the growth or refraction of nongoggled eyes after 1 week at doses of 50 $\mu$g (the dose with the strongest effect against form-deprivation myopia) or of either 10 or 1 $\mu$g (the doses that tended to stimulate the myopic response to a goggle).

**Acute Drug Effects**

Mean baseline pupil diameter measured 2.4 ± 0.5 mm. Two hours after injection, each of the drugs induced some pupillary dilation (change from baseline: 200 $\mu$g chlorisondamine, 0.8 ± 0.1 mm, $P < 0.01$; 50 $\mu$g mecamylamine, 0.3 ± 0.1 mm, not significantly changed; 1 $\mu$g mecamylamine, 0.8 ± 0.2 mm, $P < 0.05$; 5 $\mu$g methyllycaconitine, 0.4 ± 0.2 mm, not significantly changed; 50 $\mu$g dihydro-β-erythroidine, 1.0 ± 0.1 mm, $P < 0.01$). Although dilated, the pupils in each group still constricted in response to light but were sluggish. By 24 hours, the pupil had returned to normal in all but two groups (change from baseline: chlorisondamine, 0.7 ± 0.2 mm, $P < 0.05$; mecamylamine, 50 $\mu$g, 0.4 ± 0.1 mm, $P < 0.05$). None of the drug applications had a significant effect on refraction at either
Table 1. Post Hoc Pairwise Comparisons of Drug Effects: Tukey Test

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The statistically significant (defined as \( P < 0.05 \)) post hoc pairwise comparisons by the Tukey test are shown for each condition and drug for which the ANOVA identified a treatment effect (see text and Figures 1 to 3).

2 or 24 hours. By ultrasoundography, chlorisondamine induced a 0.16 ± 0.04 mm (\( P < 0.05 \)) reduction in axial length and a 0.20 ± 0.07 mm (\( P < 0.05 \)) reduction in posterior chamber depth at 2 hours, each of which returned to baseline at 24 hours; chlorisondamine also reduced lens thickness by 0.12 ± 0.04 mm (\( P < 0.05 \)) at 2 hours and by 0.16 ± 0.05 mm (\( P < 0.05 \)) at 24 hours. None of the other drugs influenced the ultrasound measurements.

Pathology

With chlorisondamine, 200 µg every other day, gross inspection of the eye cup of most of the goggled eyes (4/5) and all the never-goggled eyes (\( n = 5 \)) showed mild-to-marked mottling and depigmentation of the midperipheral fundus; a variably sized geographic area appeared relatively spared or normal in the central fundus region. The tissue sections revealed marked disruption and clumping of cells of the retinal pigment epithelium (RPE) in regions corresponding to the peripheral depigmented areas (Fig. 4A). Pigment-containing cells, presumably macrophages, were occasionally noted in the outer retina, and outer segments were sometimes disrupted overlying the disrupted epithelium. The retina otherwise appeared intact. Presumed inflammatory cells infiltrated the peripheral choriocapillaris beneath the most involved areas of the RPE, but the choroid was otherwise unaffected. The central regions of these eyes showed either normal histology or less marked changes (Fig. 4B). The goggled eye treated with 200 µg chlorisondamine that had a normal gross examination also exhibited normal histology. Of the goggled or nongoggled eyes treated with chlorisondamine, 100 µg every other day, gross inspection of the eye cups showed either a normal fundus or only mild peripheral pigmentary changes. Some eyes had normal histology, some showed a single large, smooth hyper-pigmented inclusion within a rare RPE cell as the sole detectable histologic change, and some had a small isolated peripheral patch of the marked RPE/choriocapillaris pathology as described above. Importantly, the growth and refractive responses of goggled or open eyes to chlorisondamine was not clearly related to the degree of retinal histopathology (data not shown).

The retinas of goggled and nongoggled eyes treated daily with either 200 or 50 µg mecamylamine were indistinguishable grossly or histologically from vehicle-treated control eyes.

Figure 3. Effects of chlorisondamine on nongoggled eyes. Unilateral administration of chlorisondamine to eyes of never-goggled chicks shifted overall refraction toward hyperopia (ANOVA on ranks: \( P = 0.004 \)), reduced axial length (ANOVA on ranks: ultrasound, \( P = 0.03 \); calipers, \( P = 0.03 \)), and inhibited the axial expansion of the vitreous cavity (ANOVA on ranks: \( P = 0.01 \)). For the results of pairwise comparisons by the Tukey test, see Table 1. \( n = 9 \) to 20 chicks per group. The data are illustrated as the difference between the drug-treated and contralateral vehicle-treated eye (mean ± SEM).
DISCUSSION

Nicotinic antagonists affect eye growth in young chicks, influencing both the development of form-deprivation myopia and normal eye growth. The biological effects are complex, with multiphasic dose–response curves. The responses vary between the drugs and differ from other agents altering chick eye growth.

Nicotinic Effects on Form-Deprivation Myopia

The two nonselective nicotinic antagonists have the greatest effects on experimental myopia. At the higher doses, chlorisondamine inhibits myopia by reducing vitreous cavity and axial elongation of the goggled eye. The increases in axial and vitreous cavity lengths of goggled eyes at the lower doses of chlorisondamine are not significantly different from control eyes but may be noteworthy, given the mecamylamine responses. Mecamylamine also inhibits the myopic refractive error and excessive axial growth of goggled eyes but with most activity at the intermediate dose of 50 μg and less efficacy at higher drug levels. At lower doses, mecamylamine exaggerates the growth response to goggle wear; this is most evident in the vitreous cavity measurements but also is suggested by the trends in refraction and axial length. Other general drug classes retard experimental myopia in chicks, only a few individual drugs have previously been noted to stimulate the goggle response but at higher doses than those at which nicotinic antagonists increase the growth of goggled eyes.

Nicotinic antagonists exert a distinct effect on the vitreous cavity shape of form-deprived eyes. In those prior investigations that examined both the axial and equatorial dimensions of the vitreous cavity after drug administration, vitreous cavity enlargement was more effectively suppressed in the axial than in the equatorial dimension by agents pharmacologically active against experimental myopia. This differential pharmacologic activity in the axial and equatorial dimensions of the vitreous cavity has supported the concept that vitreous cavity shape per se is a biologically regulated variable. In contrast to these other agents, nicotinic antagonists effectively suppress equatorial expansion of the vitreous cavity at the same doses active against axial elongation. The inhibition of overall vitreous cavity expansion by nicotinic antagonists may identify either a mechanism or a level of action in the neural regulation of eye growth that is different from other agents acting preferentially in the axial dimension.

Nicotinic Effects on Open, Nongoggled Eyes

Besides effects on goggled eyes, chlorisondamine inhibited eye growth when given to nongoggled eyes. This effect was manifest by reduced axial expansion of the vitreous cavity, a shorter overall axial length and a hyperopic shift in refraction, the latter likely a consequence of the reduced ocular size. This effect of chlorisondamine is distinguished from other pharmaco-

![Histopathologic effects of chlorisondamine](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932912/ on 11/24/2018)
cologic agents affecting experimental myopia. Dopaminergic agonists, muscarinic antagonists, opiates, and basic fibroblast growth factor all inhibit form-deprivation myopia; with the exception of muscarinic antagonists, none have altered growth when given to nonoccluded eyes, possibly because the visually driven eye growth mechanism predominates over any drug effect. Inhibition of nonoccluded eye growth by chlorisondamine may indicate a nicotinic receptor(s) resides at a critical juncture in the pathway linking vision and emmetropization. Alternatively, an independent action at the RPE may underlie the open eye effects of chlorisondamine because mecamylamine neither induced the RPE/choriocapillaris pathology nor altered the growth of nongoggled eyes.

Other neurally active agents have influenced eye growth when given locally to open, nongoggled eyes of chicks, but their effects on ocular anatomy have differed markedly from that of chlorisondamine. In contrast to the hyperopic refractive shift and inhibition of vitreous cavity growth from chlorisondamine, these other agents most commonly induce myopia and complex morphologic patterns of eye enlargement, with different actions on the anterior and posterior segments. Also, these other agents typically are established neurotoxins and include kainic acid, quisqualic acid, colchicine, and N-methyl-o-aspartate at toxic but not at pharmacologic doses. Unlike the myopia arising from these toxins, tetrodotoxin does induce a hyperopic refractive shift; but it also causes anterior chamber flattening and vitreous cavity elongation, morphologic influences quite different from the general eye growth inhibition after chlorisondamine. Further, postulating a mechanism for the tetrodotoxin effect is complicated by its action not only to block sodium channels and neurotransmission in ganglion cells but also to block sodium channels in many nonexcitable ocular cells, including lens, ciliary epithelium and RPE, and corneal endothelium.

Pathologic Effects of Chlorisondamine

Chlorisondamine but not mecamylamine induced RPE layer degeneration and a cellular infiltrate in the underlying choroid (Fig. 4). This drug effect was dose dependent and more prominent in the peripheral regions of the retina. Chlorisondamine previously has been noted to exert a toxic effect in neonatal rat liver as assessed by tissue enzyme activity, but there are no prior reports of histopathologic changes with this drug or toxic effects on neural tissue. In brain, chlorisondamine accumulates in regions with nicotinic receptors. A proportion of chlorisondamine to accumulate in RPE cells might in part underlie its histopathologic effect. The apparent dissociation of the RPE and eye growth effects in individual birds receiving chlorisondamine (see Results) and the lack of evident RPE alterations with mecamylamine, another effective agent against myopia, each indicate that the antimiopaya activity of nicotinic antagonists is not dependent on gross RPE disruption per se but more likely involves pharmacologic activity at nicotinic receptors.

Acute Drug Effects on the Eye

Eyes were examined 2 and 24 hours after administration of selected doses of each nicotinic antagonist to learn if any acutely influenced the tone of the intraocular muscles. Each drug induced some mydriasis, reversible to light; presumably, any cycloplegic effect also was partial. These in vivo experiments do not distinguish whether these drugs cross-reacted to the nicotinic receptors on the chick intraocular muscles and directly induced relaxation or whether they acted primarily on ciliary ganglion receptors after diffusion out of the eye and only indirectly affected muscle tone. Regardless, these drugs neither shifted refraction acutely nor uncovered any basal accommodative tone under the conditions of the experiments. Only chlorisondamine acutely altered ocular dimensions by ultrasound, transiently reducing axial and vitreous cavity lengths at the 2-hour but not at the 24-hour reading. Further, only chlorisondamine influenced lens thickness, reducing it and likely increasing the focal length; if any increase in lens focal length had modulated development of open eyes receiving this drug daily, it would have stimulated eye growth and not inhibited it (Fig. 3). Because all measurements of drug influences on eye growth were made 24 hours after the last dosing, none of the observations on growth or refractive development can be explained by an acute drug effect, muscular or otherwise, on refraction or eye component measurements.

Nicotinic Receptor Subtypes and the Eye

Each of the 16 different known nicotinic acetylcholine receptor subtypes are composed of five subunits to form an acetylcholine-gated cation channel. The receptors fall into three general classes: a muscle class and two neural classes. The muscle types exist in only two forms: a fetal and an adult form, each with α1 subunits and other subunits specific for muscle receptors. One class of neuronal receptors binds α-bungarotoxin and is composed of α7, α8, or α9 subunits, often as homomeric receptors. The other class of neuronal receptors does not bind α-bungarotoxin and is formed from combining α2, α3, α4, or α6 subunits with β2 or β4 subunits. α5 and β3 may associate with these heteromeric receptors as a third or fourth kind of subunit. Rapid desensitization and limited availability of selective drugs suited for in vivo studies have impaire defining physiologic functions for these biochemically defined receptor subtypes.

The chick retina contains several classes of cholinergic neurons. Besides several subtypes of muscarinic acetylcholine receptors, chick retinal neurons express a multiplicity of nicotinic acetylcholine receptor subunits, including α3, α6, α7, α8 and β2, β3, β4. The cellular patterns of the neural localizations of nicotinic receptors in chick retina are complex. The chick ciliary ganglion similarly is enriched with a diversity of nicotinic receptor subtypes that include the α5 subunits that typifies autonomic ganglia, α5, α7, β2, or β4 subunits, with both synaptic and extrasynaptic localizations.

Potential Site(s) of Action of Neural Nicotinic Antagonists

It seems most likely that nicotinic antagonists modulate eye growth by an action on nicotinic neural receptors and not the nicotinic receptors of the chick striated intraocular muscles. Although we did not assess accommodative capacity, each agent studied here displays relatively low affinity at muscle receptors compared with neural receptors, is typically used for studying neural nicotinic receptors, and demonstrates sufficient lipophilicity to be useful in the central nervous system. The least effective antimyopia agent, dihydro-β-erythroidine, was at least as potent a mydriatic at the 2-hour measurement time as chlorisondamine, the most effective antimyopia agent; thus, the mydriatic and growth effects are dissociated by these two agents.

The experiments with the relatively selective nicotinic receptor subtype antagonists also are consistent with a neural site of action. Methyllycaconitine blocks somewhat selectively nicotinic receptors containing the α7 and α8 subunits. Although its antimyopia efficacy was less pronounced than that of either chlorisondamine or mecamylamine, its activity still is consistent with the involvement of α-bungarotoxin-sensitive nicotinic neuronal receptors. Dihydro-β-erythroidine shows a different specificity profile; it is an antagonist with relatively higher affinity for nicotinic receptors with the α3 or α4 sub-
units and particularly α3β2-containing receptors. Dihydro-β-erythroidine was the least active of the agents studied against form-deprivation myopia. These profiles are consistent with the limited involvement of these α-bungarotoxin-insensitive nicotinic neuronal receptors. Based on the present understanding of the nicotinic receptor subtypes in the chick, the drugs available for the present study are insufficient to establish unambiguously a retinal or ciliary ganglion site of action or to define the nicotinic receptor subtypes responsible for the activities of the drugs used. In addition, some nonexoncetible cells recently have been found to express nicotinic receptors, but no data are available for the chick eye. As indicated in the Introduction, much evidence argues in support of the retina and against a central role for accommodation and the ciliary ganglion in eye growth control; it seems reasonable to hypothesize the retina as the target site for nicotinic antagonists in altering eye growth, pending further clarification of many of the issues raised here. The pathologic changes after chlorisondamine suggest that the RPE may be a target for nicotinic antagonists and identify a need to seek nicotinic receptors in this epithelial layer.

The complex dose-response curves for these drugs also do not now permit unambiguous interpretation. Each of the drugs except chlorisondamine tended to lose its antimyopia effect at the highest doses tested. Mecamylamine in particular exaggerated the myopic response to form deprivation at the lower drug doses. Whether low dose chlorisondamine similarly exaggerated myopic eye growth is unclear; the statistics do not support such a conclusion, but the refractive values, axial lengths, and vitreous cavity dimensions shift in that direction. Multiple subtypes of nicotinic receptors may participate in eye growth regulation. The response differences between the drugs may reflect different subtype affinities, and multiphasic dose-response curves may follow differential dose-dependent activation of involved receptor subtypes. Alternatively, multiple neural structures may be involved, perhaps differentially affected because of receptor populations or drug pharmacokinetic properties.

Summary

Based on the action of neurally active antagonists, nicotinic receptors are involved in eye growth control. In goggled eyes of chicks, nicotinic antagonists inhibit axial growth and reduce the myopic shift in refraction. Unlike other drugs active against form deprivation that inhibit vitreous cavity growth preferentially in the axial dimension, nicotinic antagonists reduce the overall expansion of the vitreous cavity. At low doses, they also may accentuate the myopic response to google wear. Chlorisondamine also inhibits the growth of nongoggled eyes and shifts the refraction toward hyperopia, but pathologic changes with this drug complicate interpreting its effects. Although activity at other sites cannot be completely excluded, a site of action at the neural retina or RPE is consistent with the effects of these drugs.

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