Role of the Pineal Gland in Ocular Development of the Chick in Normal and Constant Light Conditions

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PURPOSE. To evaluate the role of the pineal gland in development of the chick eye in normal and constant light (CL) conditions.

METHODS. Chicks (Gallus gallus) were raised in either a 12-hour light–dark cycle (12L/12D) or in CL, with or without opaque, removable hoods that covered the top of the head for 12 hours each day. An additional group was raised with opaque eye occluders over the right eye for 12 hours daily. Half of the chicks in each group had their pineal glands surgically removed at 3 to 6 days after hatching. Corneal curvature was measured with keratometry, anterior chamber depth with ultrasound, and refraction with infrared photoretinoscopy.

RESULTS. Pinealectomy does not affect the development of the chick eyes either in 12L/12D or CL. Covering the right eyes provided the same amount protection against CL’s effects on the corneal curvature of both eyes, with or without pinealectomy. Pinealectomized chicks were not protected from CL’s effects by 12L/12D head covers. A similar pattern of responses was obtained for refraction and anterior chamber depth.

CONCLUSIONS. Although 12L/12D covering of the pineal gland can protect chick eyes from CL’s effects (corneal flattening, shallowing of the anterior chamber, and hyperopia), the pineal gland does not appear to be necessary for normal growth in 12L/12D conditions, and its absence does not affect eye growth in CL conditions, with or without hoods or occluders. Pinealectomy does not influence the protection of an eye exposed to CL that is afforded by covering the other eye in a 12L/12D cycle. (Invest Ophtalmol Vis Sci. 2006;47: 5132–5136) DOI:10.1167/iovs.05-0671

O ur previous studies have shown that raising chicks in constant light (CL) can lead to the development of a high degree of hyperopia in newborn chicks, a severe flattening of the cornea, shallowing of the anterior chamber, and elongation of the vitreous chamber in as short a time as 2 weeks. Weiss and Schaeffel observed diurnal changes in ocular growth, with more growth during the day and even shrinkage of axial length during the night. Further they observed that these diurnal growth rhythms of the eye disappeared in chicks exposed to CL, leaving the eye in a continuing growth state. Nickla et al. reported diurnal changes in intraocular pressures (IOPs) of chicks, and found that these changes paralleled the diurnal ocular growth changes. Our previous studies have also shown that the diurnal IOP is high during the day and low at night, with a difference between day and night of 4 mm Hg.

However, after 5 days of exposure to CL, the normal rhythm of IOP disappears, and the mean IOP averaged over a 24-hour period is significantly lower than normal (Li T, et al. IOVS 2002;43:ARVO E-Abstract 197). In addition to the suppression of the IOP rhythm, lower melatonin concentrations and smaller amplitudes of its diurnal rhythms have been found in the retina, pineal gland, and blood of chicks reared in constant illumination compared with those in a 12-hour light–dark (12L/12D) cycle (Li T, et al. IOVS 2000;41:ARVO Abstract 690).

We have shown that exposure of the pineal gland of chicks to a 12L/12D light rhythm, while the eyes and remaining body are exposed to CL, significantly reduces the effects of CL on the growth and refraction of the chick eye. However, if the pineal gland and one eye are exposed to CL while the other eye is in a 12L/12D cycle, the exposed eye is better protected against CL’s effects. Yet more protection is achieved if both eyes are covered in a 12L/12D cycle, in which case the eyes grow almost normally. In addition, we have shown that daily intramuscular injections of melatonin, or melatonin eye drops, applied during subjective nighttime can prevent CL’s effects (Li T, et al. IOVS 1999;40:ARVO Abstract 847). These results suggest that the pineal gland, or either or both of the two eyes, when exposed to a 12L/12D cycle, all produce melatonin rhythms that provide protection against CL’s effects. Furthermore, the exposure of the pineal gland to CL, which suppresses the melatonin rhythm of the gland, may, in regard to protection, be equivalent to removing it. This raises the possibility that we wanted to test in the experiments reported herein—namely, that the pineal gland may not be necessary for the normal growth of the eyes or for the protection of one eye by the other when the first is exposed to CL. Our strategy was to compare corneal curvature, axial chamber depth, and refraction in normal and pinealectomized chicks that had been raised in 12L/12D and CL conditions. Under CL, the chicks were to be raised with or without covering one or both eyes or the head, in a 12L/12D rhythm.

METHODS

Animals and Treatments

All animal care and work performed in this study conformed to U.S. Department of Agriculture standards and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, in a protocol approved by Cornell’s Institutional Animal Care Committee.

Chicks (Gallus gallus, Cornell K Strain) were raised in either a 12L/12D cycle (normal group, N) or in CL, with (hooded group, HC) or without (constant light group, CL) opaque, removable hoods that covered the top of the head for 12 hours each day. The average ambient illumination level in the avairy was 700 lux on top of the brooders. The illumination was supplied by fluorescent lamps (40 W, cool white; Sylvania, Danvers, MA). The chicks were raised in temperature-controlled brooders (33 ± 0.5°C). Food (Agway, Shippensburg, PA), crop gravel, and water were provided ad libitum. An additional group was raised with opaque eye occluders over the right eye for 12 hours per day (RC group; right eye covered). There were 12 to 16 chicks in each group. Half of the chicks in each group had their pineal glands surgically removed (P; pinalectomized; HC/P; head covered,
with pinealectomy). The rest of the chicks in the same groups had sham operations as control subjects.

Surgery

The chicks were pinealectomized 3 to 6 days after hatching. For surgery, the chicks were anesthetized with ketamine (4 mg/100 g of body weight) and xylazine (0.8 mg/100 g of body weight). The feathers were removed from the head over an area of approximately 1.5 cm in diameter before the skin was incised to expose the skull. According to the location of the chick pineal gland, a 1-cm horizontal incision along with middle line on the chick head was made, to expose the skull. With a corneal–scleral punch, a 2.5 mm² piece of parietal skull was removed, exposing an area of the brain that is anterior to the cerebellum and posterior to the cerebrum in the midline of the pineal body. The pineal body was removed with an iris scissors. Before finishing the surgery, several sutures were made with 4-0 absorbable surgical sutures in both the dura mater encephali and the skin. After surgery, the animals were placed a separate warm box until they recovered and regained equilibrium. The animals then were checked two to three times daily for first 3 days after surgery to monitor postsurgical recovery. The chicks were kept in the experimental condition for 3 weeks after surgery.

Measurements

After 3 weeks of experimental treatment, measurements were made on all the animals while they were conscious. The refractive states and radii of corneal curvature were measured noncycloplegically by infrared (IR) photoretinoscopy and IR keratometry, respectively. For refraction, a neutralizing lens technique was used (just as in conventional retinoscopy) at a distance of 0.5 m. The most hyperopic value beyond which the retinal reflex was reversed was taken to be the resting refraction (taking into account the two-diopter working distance). Radius of corneal curvature was measured with an IR photokeratometer composed of eight IR LEDs arranged in a circle 298 mm in diameter that projected light of adjustable intensity onto the cornea of the chick. The axial lengths, lens thicknesses, and anterior and vitreous chamber depths were measured by A-scan ultrasonography (3M-Biosound; Biosound Easote, Indianapolis, IN). The ultrasound probe (10 MHz) was extended with a 10-mm length of soft rubber tubing filled with ultrasound transmission gel (Aquasonic; Parker Laboratories, Fairfield, NJ). The open end of the tube was placed on the corneal surface near the optic axis. Proparacaine HCl (0.5%) was used as a corneal anesthetic. All the measurements were performed as described in Li et al. When both eyes received the same treatment, observations showed that the effects on the two eyes were virtually identical, and so we reported results only for the right eye.

Statistical Tests

An analysis of variance with the Fisher protected least significant difference test (PLSD) was used for comparing the differences between groups (Statview software; Abacus Concepts, Berkeley, CA).

RESULTS

Pinealectomy on the Chicks in a 12L/12D Cycle Versus CL

Figure 1 shows that CL induced the same degree of corneal flattening (Fig. 1A), decrease in anterior chamber depth (Fig. 1B), and hyperopic refraction (Fig. 1C) in the chicks irrespective of pinealectomy. Pinealectomy seems to have had no significant effect (NS, $P > 0.05$) in the experiments in which eyes were covered in a 12L/12D cycle. There are no significant differences in ocular growth (corneal curvature, anterior chamber depth, lens thickness, vitreous chamber depth, and axial length) between pinealectomized and nonpinealectomized chicks after 3 weeks in either N or CL rearing conditions. As has been observed, the vitreous chamber depths were significantly enlarged ($P < 0.005$) in chicks raised in CL, with or without pinealectomy. Pinealectomized chicks did not show significant differences in the radius of corneal curvature (A), anterior chamber depth (B), or refractive error (C) compared with those of nonpinealectomized chick reared in either normal 12L/12D or CL.
The Effects of Covering One Eye in CL, with or without Pinealectomy

Covering the right eye for 12 hours daily blocked CL’s effects on ocular development (corneal curvature and refraction, Figs. 2A, 2C) by 80% on covered eyes and 50% on fellow eyes exposed to CL. Again, pinealectomy seemed to show no significant effects in the eye covering experiment (Fig. 2). Right-eye–covered chicks provided the same degree of protection against CL’s effects on the radius of corneal curvature (Fig. 2A), anterior chamber depth (Fig. 2B), and refractive status (Fig. 2C) of chicks, irrespective of pinealectomy, after 3 weeks of CL rearing.

The Effects of Covering the Pineal in CL, with or without Pinealectomy

Furthermore, as expected, head-covered, pinealectomized chicks did not exhibit the protection against CL’s effects that nonpinealectomized, head-covered chicks did (Fig. 3). Rhythmically covering the heads of chicks that had undergone pinealectomy made no significant differences (NS) in radius of corneal curvature (Fig. 3A), anterior chamber (Fig. 3B) depth, and refractive error (Fig. 3C) compared with those in the CL control group, whereas rhythmically covering the heads of nonpinealectomized chicks led to almost full CL protection in the radius of corneal curvature (Fig. 3A) and refraction (Fig. 3C), compared with those in N (12L/12D) conditions, and significant (P ≤ 0.0001) large protection in anterior chamber depth (Fig. 3B), compared with those in CL.

DISCUSSION

Implication of the Results

We found no significant differences in corneal radii of curvature, anterior chamber depths, or refractive errors in chicks raised with or without pinealectomy in 12L/12D conditions. From these results it appears that the pineal gland is not necessary for the normal growth of the cornea and implies that the eyes themselves are capable of supplying the melatonin rhythm necessary for their growth. Moreover, we found no significant differences in these measurements when the two groups of chicks were raised in CL. This implies that a chick with its pineal gland and eyes in CL is essentially the same as a chick without a pineal gland—that is, that CL suppresses the pineal melatonin rhythm to the point that it is ineffective for protection. It should be noted that, whereas in CL the melatonin rhythm in the pineal gland, plasma, and retina is suppressed, the concentration of melatonin in the pineal tissue remains high relative to that in the plasma and tissues (Li T, et al. IOVS 2000;41:ARVO Abstract 690).

We found that when chicks were raised in CL and one of their eyes was patched in a 12L/12D cycle, there was no difference in the protection against CL’s effects between normal and pinealectomized chicks. The protection afforded by the patch to both the patched eye and the fellow eye was not affected by the presence or absence of the pineal body. This implies that the protection afforded by patching one eye to itself or to the fellow eye is not brought about by the reflex stimulation of the pineal body and its secretion of melatonin.

Elsewhere, we have discussed possible mechanisms for the protection of an eye exposed to CL from a fellow eye that experiences a 12L/12D rhythm. The protection most likely arises from a diurnal melatonin rhythm in the protected eye, as we know that diurnal applications of melatonin can protect eyes exposed to CL. The failure of full protection of an eye exposed to CL by a fellow eye that experiences a 12L/12D rhythm is most probably due either to a reduction of the...
amplitude of the melatonin rhythm in the CL-exposed eye (as opposed to its fellow eye’s rhythm amplitude), or to a suppressive effect of CL itself on the generation of the melatonin rhythm of the eye exposed to CL.7 We do not know the origin of the melatonin’s protection of the eye exposed to CL. If the pineal gland is intact, it may well provide some portion of the protecting melatonin rhythm via the circulation. These experiments show that the melatonin need not come from the pineal gland, as the protective effects are virtually the same, with or without the pineal gland. Without the pineal gland, the protective melatonin rhythm may come, via the circulation or diffusion, either from the fellow eye or from the exposed eye itself, via a driving signal to the melatonin oscillator of the CL-exposed eye.

It should be noted that, in this experiment, the protection afforded the patched eye against CL’s effects was greater than that of the fellow (nonpatched) eye, regardless of whether the chick was pinealectomized. This may be explained by the fact that the 12L/12D patched eye had a melatonin rhythm, generated in the eye itself, of an amplitude sufficient to provide it with full protection, whereas the fellow eye did not.

Although these pinealectomy experiments show that the pineal gland is not necessary for the normal growth of the eye or for the protection of one eye by its fellow, the third set of experiments, in which normal and pinealectomized chicks were raised in 12L/12D and in CL with or without their heads being covered in a 12L/12D cycle, demonstrated that a light-dark rhythm imposed on the pineal gland can provide significant protection to the two eyes against CL’s effects. Thus, it appears that the pineal gland, although not absolutely necessary for normal growth or protection of the eyes when one or both receive a diurnal light input, is, nonetheless, one of three melatonin-secreting oscillators, and it can assume the role of the ocular oscillators when these are blocked by CL.

Evidence of the Efficacy of Pinealectomy

Last, we have shown that the head cover protection against CL’s effects depends on the presence of the pineal gland, and, as demonstrated in our previous experiments, that the pineal itself can protect the eyes against CL’s effects.

Relation to Prior Work

Some of our present observations may be explained by the experiments of Osol et al.,8 who found that pinealectomy suppresses plasma melatonin levels 60 hours after the operation, but that these levels recover to within 38% to 70% of normal 6 weeks after pinealectomy. It has also been found that pinealectomy increases retinal melatonin concentrations by 62% to 80% in both light and dark portions of the cycle in chicks raised in a 14/10-hour light-dark regimen, or in chicks raised in CL. Osol et al.9 concluded that retinal melatonin production most probably compensates for the loss of pineal melatonin. This finding explains our present observation that pinealectomized chicks can be protected from CL, presumably because a diurnal melatonin rhythm can be established in one or both retinas. This implies that, although the pineal melatonin rhythm may play a role in regulating the growth of the chick eye, it could be compensated for by the increased retinal melatonin levels produced through covering the eye 12 hours a day. Our results suggest that the pineal gland is also not necessary for intraocular communication in protecting against CL’s effects on the eye exposed to CL while the other eye is covered 12 hours daily. The nature of this protective communication could be due to neuronal, hormonal, or optical pathways, or some combination of these. Regarding the optical pathway: it is known that light entering one eye emerges from the fellow eye or from the exposed eye itself, via a driving signal to the melatonin oscillator of the CL-exposed eye.

Gwinner12 reviewed the role of melatonin in the circadian system of birds. In reviewing the data on the effect of pinealectomy on free running locomotor activity in sparrows, finches, pigeons, Japanese quail, and chickens, he noted strong species differences. In contrast to its effect in other species,
Pinealectomy had no effect on the rhythms of quail and chickens. Gwinner concluded that the pineal gland may not be the only circadian pacemaker and that overt circadian rhythms may depend on a complex system of two or more interacting oscillators. The present experiments do not speak to the interaction among the known melatonin oscillators (i.e., the pineal gland and the two retinas), which we have investigated theoretically, but they do exhibit the partial redundancy and overlapping functions of these oscillators. Just as Gwinner reported with regard to locomotor rhythms, pinealectomy exhibited remarkably little effect on the growth of the eye in N or CL conditions.

**Conclusions**

The development of the eyes in CL and 12L/12D is unchanged by pinealectomy. Pinealectomy does not influence the protection against CL’s effects afforded by a 12L/12D covered eye to an eye exposed to CL. It was not surprising that pinealectomized chick eyes could not be protected from CL’s effects by 12 hours of daily head covering, confirming the efficacy of the pinealectomy operations. Overall, the pineal gland is a sufficient but not a necessary agent in CL’s protective effects.

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**References**

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