likely site of action is the pigment epithelium. If so, these experiments provide evidence for an effect of light on the pigment epithelium without any corresponding changes in the neural retina. Given this, it seems possible that more intense exposure may exert its damaging affect on photoreceptors by first damaging the pigment epithelium.

Key words: ERG, rat retina, c-wave, light exposure, a-wave, b-wave

References

1. Steinberg RH, Schmidt R, and Brown K: Intracellular responses to light from cat pigment epithelium: origin of the ERG c-wave. Nature 227:728, 1970.
2. Faber DS: Analysis of the slow transretinal potentials in response to light. State University of New York at Buffalo, PhD Thesis, 1969.
3. Oakley B and Green DG: Correlation of light-induced changes in retinal extracellular potassium concentration with c-wave of the ERG. J Neurophysiol 39:1117, 1976.
4. Oakley B, Flaming DG, and Brown KT: Effects of the rod receptor potential upon retinal extracellular potassium concentration. J Gen Physiol 74:713, 1979.
5. Dodt E and Echte K: Dark and light adaptation in pigmented and white rat as measured by ERG threshold. J Neurophysiol 24:427, 1961.
6. Weidner C: The c-wave in the ERG of albino rat. Vision Res 16:753, 1976.
7. Graves AL, Green DG, and Powers M: The c-wave of the electroretinogram in light- and dark-reared albino rats (Abstr). Society for Neuroscience Meeting 5:786, 1979.
8. Pautler EL and Noell WK: The slow ERG potentials in hereditary retinal dystrophy of albino and pigmented rats. Exp Eye Res 22:493, 1976.
9. Fisher L: Development of synaptic arrays in the inner plexiform layer of neonatal mouse retina. J Comp Neurol 187:359, 1979.
10. Cicerone CM and Green DG: Signal transmission from rods to ganglion cells in rat retina after bleaching a portion of the receptive field. J Physiol (Lond) 314:213, 1981.
11. Hoffert JR and Ubels JL: Retinomotor activity and the c-wave of the hypoxic trout retina. Invest Ophthalmol Vis Sci 18:756, 1979.

5β-Dihydrocortisol: Possible Mediator of the Ocular Hypertension in Glaucoma

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5β-dihydrocortisol potentiates the action of topically applied dexamethasone in raising the intraocular pressure (IOP) in young rabbits. Dexamethasone (0.06%) plus 5β-dihydrocortisol (0.1 and 1.0%) elevated the IOP 7–10 mmHg within 18 days of treatment. By contrast, 0.06% dexamethasone alone raised the IOP 3 to 4 mmHg in a similar period of time. Since 5β-dihydrocortisol accumulates abnormally in cultured cells derived from the outflow region of the eye from patients with primary open angle glaucoma (POAG), a similar potentiation in man may account for the sensitivity of these patients to the IOP raising effect of glucocorticoids. Further, this metabolite may potentiate endogenous glucocorticoids resulting in the ocular hypertension characteristic of POAG. Invest Ophthalmol Vis Sci 26:393–395, 1985

Primary open angle glaucoma (POAG) is the most common form of glaucoma and a major cause of blindness. Most patients with POAG show a marked sensitivity to the intraocular pressure (IOP) raising effects of topical glucocorticoids.[1–3] Evidence has accumulated suggesting that a glucocorticoid metabolite may play a role in the ocular hypertension and glucocorticoid sensitivity found in this disorder.[4–6]

Cells cultured from specimens obtained by trabeculectomy from patients with POAG (TM$_{POAG}$ cells) showed an altered pattern of cortisol metabolism leading to the accumulation of both 5α and 5β-dihydrocortisol,* intermediates not found in similar cells derived from nonPOAG patients.[4] Assays of cortisol metabolizing enzymes in homogenates of TM$_{POAG}$ cells, under optimal conditions, indicated that the accumulation of dihydrocortisol was due to alterations in two enzymes: a marked increase in cortisol Δ⁴-reductase and a decrease in 3-oxidoreductase.[5] In acute experiments, 5β-dihydrocortisol (and not 5α-dihydrocortisol) was found to potentiate the cortisol and dexamethasone-induced nuclear translocation of the cytoplasmic glucocorticoid receptor in the rabbit, an early and necessary event in steroid hormone action.[6]

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* Generic names of steroids: 5α (or 5β)-dihydrocortisol, 11β,17,21-trihydroxy-5α (or 5β)-pregnane-3,20-dione; dexamethasone (21-phosphate), 9-fluoro-16α-methyl-4β,17α,21-trihydroxy-1,4-pregnadiene (-21-phosphate).
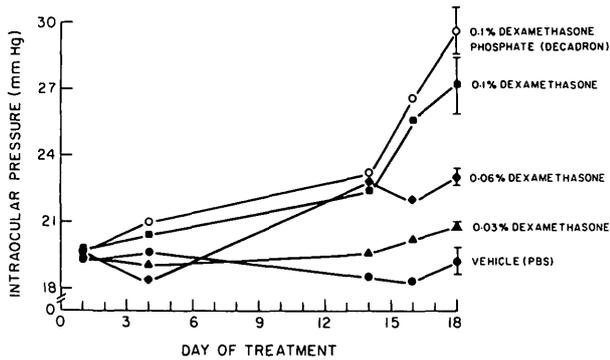
Fig. 1. Dose response of intraocular pressure (IOP) to dexamethasone in the rabbit. Intraocular pressure was measured in the rabbit after topical (ocular) administration of vehicle (phosphate buffered saline, PBS), varying concentrations of dexamethasone and dexamethasone phosphate (Decadron). Each point shown represents the mean IOP of four to six animals. The standard deviation of the means are shown for the last day of the experiment (day 18). Analysis of variance using the Bonferroni post-test[8] showed that the IOP of the animals treated with 0.1% dexamethasone phosphate, 0.1% dexamethasone, and 0.06% dexamethasone were elevated significantly from the vehicle control (PBS) ($P < 0.01$, 0.01, and 0.05, respectively). The small elevation in IOP seen with 0.03% dexamethasone was not statistically significant.

We now report that *chronic* topical application of 5β-dihydrocortisol potentiates the intraocular pressure raising effect of threshold levels of dexamethasone in rabbits. This provides direct evidence in support of the hypothesis that 5β-dihydrocortisol mediates the sensitivity of POAG patients to exogenous glucocorticoids. Further, this metabolite may potentiate en-
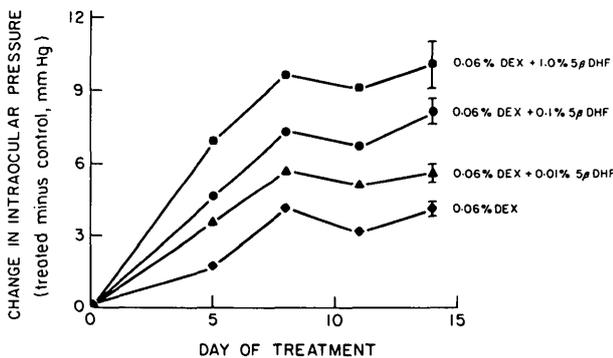

Fig. 2. Potentiation of the intraocular pressure (IOP) raising effect of a threshold level of dexamethasone (0.06%) by 5β-dihydrocortisol in the rabbit. The values are plotted as a change in the IOP relative to a vehicle control. Each point represents the mean IOP of four to six animals. The standard deviation of the means are shown for the last day of the experiment (day 14). Analysis of variance using the Bonferroni post test[8] showed that the IOPs in the animals treated with 0.1 and 1.0% 5β-dihydrocortisol together with 0.06% dexamethasone were elevated significantly from that found with 0.06% dexamethasone alone ($P < 0.05$ and 0.01, respectively). The small elevation in IOP seen with 0.01% 5β-dihydrocortisol with 0.06% dexamethasone was not statistically significant.
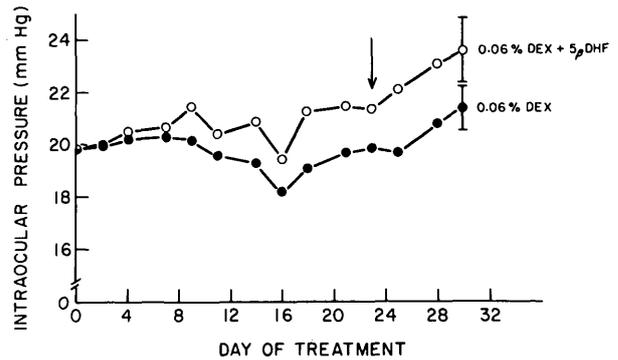

Fig. 3. Unilateral potentiation of a threshold level of dexamethasone on intraocular pressure (IOP). Eight animals were treated with 0.06% dexamethasone in one eye and 0.06% dexamethasone with 0.1% (and later 0.2%) 5β-dihydrocortisol in the other eye. Analysis of variance using the Bonferroni post test[8] showed a significant difference ($P < 0.05$) in IOP starting on day 16. The arrow indicates the day where dihydrocortisol was increased to 0.2%.

dogenous glucocorticoids resulting in the ocular hypertension characteristic of this disease.

Materials and Methods. Young New Zealand Albino rabbits weighing <2 Kg were used to test for biologic activity of 5β-dihydrocortisol since these animals have been reported to be consistently sensitive to the IOP raising effects of glucocorticoids.[7] Animals were treated by placing 25 μl of the test solutions in each eye four times a day. Intraocular pressure was measured several times a week between 8 to 10 AM with an Alcon pneumotonometer (O.C.V.M. from Digilab Division of Bio-Rad) after addition of a topical anesthetic (tetracaine). A single mean value was used for each animal. Each group contained from four to six animals and the data reported are the mean IOP of each group. In one experiment with eight animals threshold levels of dexamethasone were administered bilaterally and 5β-dihydrocortisol unilaterally, four times daily. The steroids were suspended in phosphate buffered saline (PBS) by homogenization with a Teflon pestle. This produced a fine suspension of the steroids that minimized corneal irritation. The experiments were carried out in a masked fashion and conformed to the ARVO Resolution on the Use of Animals in Research.

Results and Discussion. Figure 1 shows the average IOP in groups of animals treated with varying concentrations of dexamethasone or with vehicle alone as a function of time. The animals treated with vehicle showed less than 2 mmHg variation in IOP during the entire course of the experiment. The dexamethasone-treated animals showed a dose response to the steroid with 0.1% dexamethasone and its phosphate ester (Decadron) significantly (see legend) raising IOP after 2 weeks of treatment while
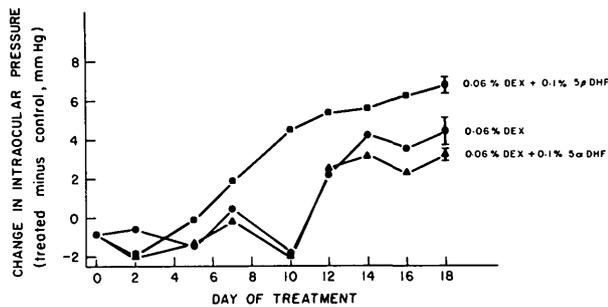
Fig. 4. Specificity of potentiation of the intraocular pressure (IOP) raising effect of a threshold level (0.06%) of dexamethasone. Each point represents the mean IOP of four to six animals. The standard deviations of the means are shown for the last day of the experiment (day 18). Analysis of variance using the Bonferroni post-test[8] showed that the administration of 0.1% 5β-dihydrocortisol with 0.06% dexamethasone produced a significant elevation in IOP starting on day 10 ($P < 0.002$). By contrast, administration of 0.1% 5α-dihydrocortisol with 0.06% dexamethasone did not result in a significant change in IOP.

0.06% dexamethasone showed a lesser but significant intraocular hypertensive response. Dexamethasone (0.03%) showed no significant elevation in IOP even after 18 days. Therefore, under these conditions of treatment, 0.06% dexamethasone was determined to be the threshold level of steroid for this response. In another experiment, varying concentrations of 5β-dihydrocortisol were given together with 0.06% dexamethasone. These results are shown in Figure 2 where the changes in IOP relative to the control group (PBS) are plotted as a function of day of treatment. Dexamethasone (0.06%) alone raised the IOP 3 to 4 mmHg within 2 weeks, similar to that seen in Figure 1. The addition of 5β-dihydrocortisol (0.1% and 1.0%) to the threshold level of dexamethasone produced a dose-related potentiation of the IOP raising effect of the steroid. Not shown are experiments in which 5β-dihydrocortisol (0.1 and 1.0%) by itself produced no change in IOP (<2 mmHg) even after 6 weeks of treatment. In another set of animals both eyes received 0.06% dexamethasone, and one eye received 0.1% (and later 0.2%) 5β-dihydrocortisol (Fig. 3). The eyes receiving 5β-dihydrocortisol had a significantly (see legend) greater IOP starting on day 16 of treatment. Thus, the potentiating effect of 5β-dihydrocortisol is, at least in part, a direct action of the metabolite on the ocular tissue. Figure 4 shows

the specificity of the potentiation. In contrast to 5β-dihydrocortisol, 5α-dihydrocortisol (0.1%) did not significantly alter the IOP raising effect of 0.06 dexamethasone. Dexamethasone (0.1%) and dexamethasone (0.06%) plus a 0.1% 5β-dihydrocortisol-treated animals failed to gain—but did not lose—weight. The vehicle treated groups showed a steady weight gain during the course of these experiments.

These data suggest that 5β-dihydrocortisol, which accumulates abnormally in cultured cells derived from the outflow region of the eye from patients with POAG, is responsible for the sensitivity of these patients to exogenous glucocorticoids. Further, this metabolite may potentiate endogenous glucocorticoids, resulting in the ocular hypertension characteristic of POAG.

Key words: 5β-dihydrocortisol, cortisol metabolism, intraocular pressure, glaucoma

References

1. Armaly MF: Effect of corticosteroids on intraocular pressure and fluid dynamics. I. The effect of dexamethasone in the glaucomatous eye. Arch Ophthalmol 70:492, 1963.

2. Becker B and Mills DW: Corticosteroids and intraocular pressure. Arch Ophthalmol 70:500, 1963.

3. Becker B: Intraocular pressure response to topical corticosteroids. Invest Ophthalmol 4:198, 1965.

4. Southren AL, Gordon GG, Munnangi PR, Vittek J, Schwartz J, Monder C, Dunn MW, and Weinstein BI: Altered cortisol metabolism in cells cultured from trabecular meshwork specimens obtained from patients with primary open angle glaucoma. Invest Ophthalmol Vis Sci 24:1413, 1983.

5. Weinstein BI, Munnangi PR, Gordon GG, and Southren AL: Defects in Cortisol Metabolizing Enzymes in Primary Open Angle Glaucoma. Invest Ophthalmol Vis Sci 1985, in press.

6. Weinstein BI, Gordon GG, and Southren AL: Potentiation of glucocorticoid activity by 5β-dihydrocortisol: its role in glaucoma. Science 222:172, 1983.

7. Knepper PA, Breen M, Weinstein HG, and Black LJ: Intraocular pressure and glycosaminoglycan distribution in the rabbit eye: effect of age and dexamethasone. Exp Eye Res 27:567, 1978.

8. Wallenstein S, Zucker CL, and Fleiss JL: Some statistical methods useful in circulation research. Circ Res 47:1, 1980.