Glucocorticoid localization by radioautography in the rabbit eye following systemic administration of $^3$H-dexamethasone. ANDRES TCHERNITCIN, EUGENE J. WENK, M. ROSARIO HERNANDEZ, BERNARD I. WEINTSTEIN, MICHAEL W. DUNN, GARY G. GORDON, and A. LOUIS SOUTHREN.

Dexamethasone was localized radioautographically in the nuclei of target cells of the rabbit eye following intravenous administration of the labeled steroid. Specifically, bound steroid was found in the nuclei of stromal and endothelial cells of the outflow pathway region. This suggests that the glucocorticoid-induced alteration in outflow facility may be mediated by specific effects in these target cells. Nuclear localization was also found in conjunctiva, iridial smooth muscle, choroidal stroma, retina, and sclera, suggesting a physiologic role for glucocorticoids in these tissues as well.

Glucocorticoid administration has been shown to increase intraocular pressure in rabbits and humans (see ref. 3 for review). A dexamethasone-binding protein, exhibiting many of the biochemical properties of a glucocorticoid receptor, has previously been demonstrated in homogenates of rabbit iris-ciliary body and adjacent corneoscleral tissue. More recently, it has been shown that this binding protein migrates from the cytoplasm to the nucleus in response to systemic administration of physiologic doses of glucocorticoids, thereby confirming the presence of glucocorticoid target cells within this tissue.

These biochemical methods, however, are not able to identify which specific cells in these tissues are hormone sensitive. Identification of the specific glucocorticoid target cells is necessary to further understand the mechanism by which these hormones affect intraocular pressure. A preliminary report from this laboratory described $^3$H-dexamethasone localization in rabbit eye tissues following both in vitro incubation and intracamerale injection of the labeled steroid. We now report on the radioautographic localization of $^3$H-dexamethasone following intravenous injection with and without a large excess of nonlabeled steroid. Systemic administration of the labeled hormone provides wide distribution to eye tissues in the absence of local trauma. These experiments permit a more accurate assessment of the physiologic distribution of glucocorticoids in ocular tissue.

Materials and methods. A radioautographic technique previously shown to be suitable for the localization of diffusible substances, including steroids, was used for this study. Seven female albino New Zealand rabbits weighing approximately 2 kg were injected with 1.5 to 1.9 mCi of 6,7-$^3$H-dexamethasone (Sp. Ac. 26.4 Ci/mmol, New England Nuclear) into the marginal ear vein. Three control rabbits were injected simultaneously with 1.9 mCi 6,7-$^3$H-dexamethasone and a 300-fold excess (8 mg) of nonlabeled steroid. After intervals ranging from 75 to 180 min the animals were anesthetized with sodium pentobarbital, and the eyes were enucleated. Tissue samples were dissected, frozen in liquid Freon over liquid nitrogen, and stored in liquid nitrogen. From each sample, cryostat sections 6 μm thick were freeze-dried in a cryo-pump (Thermovac Industries, Inc., Copiague, N. Y.) at −35°C with a pre-vacuum produced by an oil-diffusion pump backed by a mechanical pump. Freeze-dried sections were pressed onto glass slides which had previously been coated with radioautographic emulsion (NTB-3 Kodak) that had been allowed to dry. After 3 to 8 months of exposure at −20°C, radioautographs were developed for 35 to 75 sec in developer at 20°C, fixed, and then stained in hematoxylin-eosin (H&E).

Results. Radioautographs demonstrated label predominantly over the nuclei of connective tissue cells and small vessel endothelial cells in the outflow pathway region, ciliary body, and iris (Figs. 1, B, 2, A, and 3, B). There was essentially no difference in localization of label at the various time periods following injection. In addition, label was found in the nuclei of smooth muscle cells in the iris (Fig. 3, C and D). Although label was also found in the epithelial layers of the ciliary body (Fig. 2, A) and the iris, there was no apparent concentration of the silver grains on the cell nuclei. Nuclear localization of label was also seen in stromal cells and endothelial cells throughout the sclera (Figs. 1, D, and 4, B) and the choroid (Fig. 4, A). In the conjunctiva the epithelial, stromal, and endothelial cells showed nuclear localization of the grains (Fig. 4, C). In the retina, cell nuclei were labeled predominantly in the inner nuclear layer, but specific cell types in this layer could not be definitively identified with the method used. It is of interest that the concentration of the label in the lens and cornea was significantly less than in the other tissues and that no nuclear localization was found.

When control rabbits were treated simulta-
Fig. 1. Radioautographs of the outflow pathway region after 4 months exposure to emulsion. 

A, Orientation photomicrograph focused on the tissue, showing anterior chamber (AC), iris (I), Descemet's membrane (DM), spaces of Fontana (F), sclera (Sc), and ciliary body (CB). (H & E; ×235.) 

B, Nuclear localization of ³H-dexamethasone in stromal cells of region similar
Fig. 2. Radioautographs of rabbit ciliary body after 4 months of exposure to emulsion. A, Nuclear localization (arrows) of $^{3}H$-dexamethasone in stromal and endothelial cells of a blood vessel (BV). (H & E; ×660.) Note lack of nuclear localization in epithelium (E) despite high concentration of grains. B, Random distribution of silver grains, without specific nuclear localization, in ciliary body after injection of $^{3}H$-dexamethasone with an excess of nonlabeled steroid. (H & E; ×600.)

eously with a large excess of nonlabeled hormone, nuclear binding of the tritiated hormone was suppressed in all cases (Figs. 1, C and E, and 2, B).

**Discussion.** The nuclear localization of $^{3}H$-dexamethasone in specific cell types in the rabbit eye most likely reflects the binding of the steroid to the cytoplasmic receptor and its subsequent translocation to the nucleus. This is consistent with the accepted mechanism whereby steroid hormones regulate differential gene expression in the cell nucleus, although it does not preclude other mechanisms of hormone action.

The localization of glucocorticoid receptors in the outflow region indicates that stromal and endothelial cells are target cells for these hormones. This suggests that glucocorticoid-induced ocular hypertension may be mediated by a direct action of the hormones on the metabolism of these target cells. These hormones may alter the synthesis, modification, secretion, and/or degradation of polymers such as glycosaminoglycans or collagen in the outflow region of the eye. The subsequent alteration of the biochemical composition of the outflow region may result in the decrease in outflow facility which characterizes steroid-induced ocular hypertension and primary open-angle glaucoma. The absence of nuclear localization in the epithelia of the iris-ciliary body, the site of aqueous humor production, is consistent with the concept that glucocorticoid-induced ocular hypertension is not due to increased secretion of aqueous humor. The finding of nuclear localization in the conjunctiva, iridial smooth muscle, retina, choroidal stroma, and sclera suggests that glucocorticoids may have a physiologic role in these tissues as well.

In contrast to the other ocular tissues, the lens

Fig. 1. Cont'd, to rectangle 1 in A. (H & E; ×760.) C, Absence of nuclear localization in stromal cells of region similar to B after injection of $^{3}H$-dexamethasone with an excess of nonlabeled steroid. (H & E; ×760.) D, Nuclear localization of $^{3}H$-dexamethasone in stromal cells of scleral region similar to rectangle 2 in A. (H & E; ×660.) E, Absence of nuclear localization in stromal cells of scleral region similar to D after injection of $^{3}H$-dexamethasone with an excess of nonlabeled steroid. (H & E; ×660.)
and cornea are nourished by the aqueous humor rather than through the blood. Moreover, it has been shown that the concentration of glucocorticoids in aqueous humor of the rabbit, calf, and human (unpublished data) is about 1% to 10% of that found in the serum. Thus the lack of demonstration of specific binding in these tissues should not be interpreted to mean an absence of glucocorticoid target cells but rather may represent insufficient exposure of the tissue to the injected steroid during the time course of the experiment. Indeed, we have identified a glucocorticoid receptor in the calf lens by both biochemical and in vitro radioautographic techniques and in the rabbit cornea by radioautography after topical administration of the steroid; all these techniques bypass the blood-aqueous barrier.

The present study confirms our previous find-
Fig. 4. Radioautographs from the choroid, the posterior segment of the sclera, and the conjunctiva after 8 months of exposure to emulsion. (H & E; ×660.) A, Choroidal stromal cells demonstrating nuclear localization of the labeled hormone (arrows). Portion of the retina (R) is nearby. B, Scleral stromal cells and endothelial cells showing nuclear localization of silver grains. C, Nuclear localization of labeled hormone by cells of the conjunctival epithelium (E) and stroma.

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REFERENCES


Sensitivity of photoreceptors to elevated levels of cGMP in the human retina.

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When isolated human retinas were cultured in the presence of a phosphodiesterase inhibitor or dibutyryl cyclic guanosine monophosphate (dbcGMP), degenerative changes occurred which were proportional to the concentration of drug used and the period of exposure. Low concentrations of either drug did not alter retinal morphology as compared to controls. Higher concentrations provoked vesiculation of rod inner segments and rounding up of cones. Numerous pyknotic nuclei were noted in the outer nuclear layer of those preparations. Combining IBMX and dbcGMP in the same medium destroyed virtually every rod in the specimen within 8 hr of incubation. Under those conditions, cones remained structurally intact although somewhat rounded. In all treatments, cells of the inner retinal layers maintained normal morphology. Our results suggest that elevated levels of cGMP in the human retina can alter certain metabolic processes in photoreceptors, which leads to degenerative changes and cell death uniquely in rod photoreceptors.

Several inherited retinal diseases in animals are found to be associated with elevated retinal levels of cyclic guanosine monophosphate (cGMP). It is thought that the basis for these disorders is a phosphodiesterase (PDE) in the outer retina which is unable to effectively hydrolyze cGMP. For example, in rd (retinal degeneration) mice, rod photoreceptors begin to degenerate around 10 days postnatally. Elevations of cGMP occur 2 to 3 days before this time, peaking at day 10 when degenerative changes are most evident. Furthermore, the PDE in the outer retina of the rd mouse shows decreased activity for cGMP hydrolysis as compared to PDE activity in normal mice. A strain of Irish setter dogs is afflicted by a disease which has a similar etiology and, like the disease in the rd mouse, has abnormally low PDE activity and correspondingly high levels of cGMP. 4, 5

Lolley et al. 8 and Hollyfield et al. 7 utilized an in vitro system and the eye rudiment of the toad, Xenopus laevis, to directly assess the effects of high levels of cGMP on developing photoreceptor viability. When retinas were cultured in the presence of a...