Increased concentration of glucocorticoid receptors in rabbit iris–ciliary body compared to rabbit liver. GEORGE R. McCARTY AND BERNARD SCHWARTZ.

Dexamethasone binding to both rabbit iris–ciliary body and rabbit liver glucocorticoid receptors was compared by means of a batch assay in which the binding components were adsorbed to hydroxylapatite. The results showed that homogenates of both the rabbit iris–ciliary body and the rabbit liver contained a single class of high-affinity receptors. Binding affinity was virtually identical for the two receptors. A comparison of total binding sites in homogenates of the iris–ciliary body and the liver revealed that the iris–ciliary body glucocorticoid receptor was present in nearly twice the concentration of the hepatic receptor. (INVEST OPHTHALMOL VIS SCI 23:525-528, 1982.)

Pharmacologic concentrations of glucocorticoids have been shown to induce ocular hypertension in both humans1-3 and rabbits.4-6 Of particular interest have been several studies reporting that patients with ocular hypertension or open-angle glaucoma have elevated plasma cortisol levels compared with ocularly normal individuals.7-9 However, the cortisol levels observed in these patients are still within physiologic limits and do not approach hyperphysiologic or pharmacologic levels. This finding leaves unanswered the serious question of whether these hormones can have an ocular hypertensive effect at slightly elevated but still physiologic concentrations. Small elevations in plasma glucocorticoids may have an adverse effect in the eye if ocular tissues are differentially more sensitive to the effect of glucocorticoids than other glucocorticoid target tissues. A differential glucocorticoid sensitivity in mammalian ocular tissues may be associated with the following factors alone or in combination: an affinity of ocular glucocorticoid receptors for glucocorticoids which is greater than that of other glucocorticoid target tissues, a differential increase in the quantity of ocular glucocorticoid receptors compared with other glucocorticoid target tissues, a greater biologic responsiveness in ocular cells to these hormones than in other glucocorticoid-responsive cells for which the mechanism of action is at the nuclear level.

At the present time, very little is known about the differential glucocorticoid sensitivity of ocular tissues compared with other glucocorticoid target tissues. Glucocorticoid receptors have previously been characterized in the rabbit iris–ciliary body,10,11 but their properties have never been compared with receptors from other glucocorticoid tissues.

The purpose of this study was to initiate an evaluation of mammalian ocular glucocorticoid sensitivity by comparing glucocorticoid receptor binding in homogenates of rabbit iris–ciliary body with homogenates of rabbit liver.

Materials and methods

Animals. Five male New Zealand white rabbits 8 weeks of age and weighing 1.6 kg were used in each experiment.

Tissue harvesting. Each animal was painlessly killed with an intravenous injection of 50 mg/kg of sodium pentobarbital. The eyes were enucleated and the iris–ciliary bodies were dissected free and weighed. After perfusion of the liver with saline, a piece of tissue having the same weight as the removed iris–ciliary bodies was dissected from the same animal.

The following procedures were performed in parallel using the ocular and hepatic tissues isolated with the above method.

Receptor isolation. Tissues were washed in cold...

---

Fig. 1. Representative Scatchard plot comparison of 3H-dexamethasone binding between rabbit iris–ciliary body (o) and hepatic (●) glucocorticoid receptors. The equilibrium dissociation constants (Kds) were calculated from the slopes of the binding plots. The total number of binding sites (Bmax) was determined from the X-axis intercept of the binding plot.
Hanks’ balanced salt solution and blotted dry. The tissues were then suspended in a buffering solution (TEM) that contained 10 vol (10:1 v/w) of 10% glycerol containing 50 mM Tris(hydroxymethyl)ammonium, 2.0 mM tetrasodium ethylenediamine tetraacetic acid, and 10 mM 2-mercaptoethanol, pH 7.8, at 0° C. The suspensions were homogenized on ice with a Brinkman polytron. The resulting homogenates were centrifuged at 0° C for 70 min at 105,000 x g. The receptor-containing supernatants (cytosols) were diluted with TEM to a final volume equal to 16 times the original wet weight of the tissue (v/w) and placed on ice.

Kinetics of glucocorticoid binding to isolated receptors. The kinetics of binding to isolated receptors was accomplished with a modification of the method described by Pavlik and Coulson. In brief, concentrations of 3H-dexamethasone, containing or lacking a 100-fold excess of unlabeled dexamethasone, were dispensed in triplicate into 13 by 72 mm tubes. Concentrations were such that when redissolved in 200 µl, a range of final concentrations from 1.25 to 40.0 nM were produced. The ethanol vehicle was evaporated and the tubes were placed on ice. Two hundred microliters of cytosol were added to each tube. The tubes were briefly vortexed every 15 min on three successive occasions to dissolve the dexamethasone. All tubes were then incubated for 16 to 18 hr on ice in the refrigerator.

The bound and unbound 3H-dexamethasone were separated by the addition of 0.5 ml of a 100 mg/ml suspension of hydroxylapatite equilibrated in TEM with mixing. Samples were vortexed, incubated at 0° C for 10 min, and centrifuged at 1500 x g at 0° C for 5 min. The supernatants were aspirated and discarded. The hydroxylapatite pellet was washed twice with 2.0 ml of TEM at 0° C. Hydroxylapatite binds the hormone-receptor complex with high affinity, and the unbound 3H-dexamethasone was removed with the washes. The bound 3H-dexamethasone was obtained by extracting the hydroxylapatite pellet once with 1.0 ml of ethanol for 20 sec at room temperature and counting 0.5 ml by liquid scintillation spectrometry. The quantity of 3H-dexamethasone bound to the receptors was determined from the difference in 3H-dexamethasone bound between duplicate samples lacking or containing a 100-fold excess of unlabeled dexamethasone.

Scatchard plots were used to calculate the binding affinities and total number of binding sites. The values of free (unbound) hormone at each concentration were obtained by subtracting the receptor-bound dexamethasone at each concentration from the total amount added. Protein content was determined by the method of Lowry et al. with bovine serum albumin as the standard.

Results. Fig. 1 is a comparison between Scatchard plots generated for the rabbit iris–ciliary body and hepatic glucocorticoid receptor. The data show a single class of high-affinity binding sites in homogenates of both tissues. Furthermore, the binding constants (Kds) are almost identical, suggesting the presence of similar, if not identical, molecules in both tissues. Of special interest is the observation that the ocular receptor was found in higher concentration than the hepatic receptor (the total number of binding sites is determined from the X-axis intercept of the Scatchard plot).

Table I is a summary of three separate experiments in which the binding kinetics between the rabbit iris–ciliary body and hepatic glucocorticoid receptors were compared. These data support the concept that the rabbit iris–ciliary body and hepatic receptors are similar if not the same. The data also indicate that there are twice the number of receptors in homogenates of iris–ciliary body compared with homogenates from the liver of the rabbit.

Discussion. The data presented in this study are the result of an initial biochemical comparison of glucocorticoid receptors in the iris–ciliary body with glucocorticoid receptors in a well-established...
glucocorticoid target tissue—the liver. The results showed that homogenates of the rabbit iris-ciliary body contained nearly twice the concentration of glucocorticoid receptors as did homogenates of the rabbit liver. The liver is already known to be a classic glucocorticoid organ. The concentration of receptors in a given tissue may or may not be a measure of differential glucocorticoid sensitivity. Ballard et al. performed an extensive comparison of glucocorticoid receptors in many extraocular glucocorticoid-responsive tissues in the rabbit. Their data clearly showed that homogenates of tissues known to be highly sensitive to glucocorticoids, such as the thymus and spleen, contained by far the greatest concentrations of receptors in comparison with other body tissues.

Homogenates of the thymus contained five times the concentration of receptors as homogenates of the liver, while homogenates of the spleen were observed to contain two times the concentration of receptors as homogenates of the liver. Homogenates of other target organs, such as adipose tissue, brain, and kidney, contained quantities of glucocorticoid receptors comparable to the quantities observed in homogenates of the liver.

Extrapolating the findings in this study to those observed by Ballard et al. must be done with some reservation because of the differences in methods between the two studies. Nevertheless, these findings do suggest that the iris-ciliary body may be among the more glucocorticoid-sensitive tissues in the rabbit. Currently, there is not an adequate bioassay to evaluate the biologic responsiveness (e.g., the induction of specific enzymes or synthesis of other specific proteins) of the iris-ciliary body to physiologic levels of glucocorticoids. Therefore biologic confirmation of this concept is currently not readily attainable.

The observation that the rabbit iris-ciliary body and hepatic glucocorticoid receptors appear to be identical molecules is not surprising. The study performed by Ballard et al. in the rabbit showed similar binding affinities for glucocorticoids among the tissues examined. A recent study in the rat showed that the glucocorticoid receptors in homogenates of adipose, kidney, and thymus tissues were indeed identical in nature. As in the rabbit, homogenates of the rat thymus were observed to contain a much higher concentration of receptors than either homogenates of the rat kidney or of rat adipose tissues. Based on these observations, the data in the present study initially support the hypothesis that the iris-ciliary body may be among the more glucocorticoid-sensitive tissues in the rabbit. It remains to be seen whether the same is true for humans.

From the Department of Ophthalmology, New England Medical Center, and Tufts University School of Medicine, Boston, Mass. Supported by grants EY00024 from the National Institutes of Health and Training Program in the Visual Sciences, No. EY07045. Submitted for publication Aug. 13, 1981. Reprint requests: George McCarty, Ph.D., Department of Ophthalmology, Tufts University School of Medicine, 171 Harrison Ave., Boston, Mass. 02111.

Key words: glucocorticoid, receptor, rabbit, iris-ciliary body, hepatic receptor

REFERENCES
13. Pavlik EJ and Coulson PB: Hydroxylapatite "batch assay" for estrogen receptors: Increased sensitivity

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 4%) was associated with iris hypochromia, but identical treatment with a beta-adrenergic antagonist (timolol 4%) 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 demonstrated heterochromia iridis in newborn rabbits subjected to interruption of the cerebral sympathetics. In clinical practice, a Horner’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 Horner’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 pigmentation is not well understood. Sympathetic innervation appears to have no effect on iris pigment epithelial pigmentation, suggesting that sympathetic innervation influence on iris pigmentation is mediated by iris stromal melanocytes.

Since sympathetic innervation does not influence skin melanocytes, the way in which sympathetic innervation of the eye 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, 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 Laties 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 is 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 4% 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 4% (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