and its Cl permeability determine the rate of secretion. Hence increasing the gradient and/or the Cl permeability should increase the net solute secreted by the epithelium, reducing stromal thickness change nearly perfect semipermeable membrane, and should be proportional to the rate of net solute secretion.

It is possible that enhancing the Cl electrochemical potential gradient across the epithelium in vivo might also result in an increased participation of the epithelium in the regulation of stromal hydration.

The very constructive criticisms of Dr. David M. Maurice and the skilled assistance of Mr. Otto Bernegger are gratefully acknowledged.

From the Division of Ophthalmology, Department of Surgery, Stanford University School of Medicine, Stanford, Calif. Supported by Public Health Service Grants EY 00015 and EY 00051. Submitted for publication April 10, 1977. Reprint requests: Stephen D. Klyce, Ph.D., S018 Stanford Medical Center, Stanford, Calif. 94305.

Key words: epithelium, Cl transport, regulation of corneal hydration, theophylline, SO4-Ringer, Cl permeability, rabbit, cornea.

REFERENCES


Specific glucocorticoid receptor in the iris-ciliary body of the rabbit. BERNARD I. WEINSTEIN, KURT ALTMAN, GARY G. GORDON, MICHAEL DUNN, AND A. LOUIS SOUTHERN.

The cytoplasm of the iris-ciliary body of the rabbit contains a receptor capable of specifically binding dexamethasone. This binding protein has a high affinity for dexamethasone (average K, = 2.0 × 10^-10 M), a low capacity (average 4.8 × 10^-12 mol of steroid bound per milligram of protein), and extreme heat sensitivity; it exhibits a pattern of competition virtually identical to that obtained with glucocorticoid receptors from other tissues and shows characteristic physicochemical behavior in various salt concentrations. The demonstration of a specific dexamethasone receptor in the iris-ciliary body provides the first direct biochemical evidence that these tissues may function as a target organ for glucocorticoids.

A considerable amount of literature has accumulated concerning the clinical nature of the glucocorticoid-induced glaucomatous response, but few biochemical data have appeared delineating the mechanism(s) of this effect. In order to gain insight into this phenomenon, we first studied the metabolism of cortisol and other steroid hormones in the iris-ciliary body of the rabbit. This species was chosen since, as in the human eye, topical administration of glucocorticoids has been reported to elevate the intraocular pressure. The iris and ciliary body, which are involved in aqueous humor dynamics, was shown to possess a variety of steroid-metabolizing enzymes. However, in order to determine whether the iris-ciliary body represents a target tissue for glucocorticoids, it was necessary to demonstrate the presence of specific cytoplasmic glucocorticoid-binding receptors, which are required for the steroid to exert its biological effect.

The present study is the first demonstration of the presence of such glucocorticoid receptors in the iris-ciliary body of the rabbit.

**Materials and methods**

Preparation of tissue. New Zealand white rabbits, 2 to 3 kg., were sacrificed by injection of a bolus of air into the ear vein. The eyes were enucleated, and the iris-ciliary body was removed in one piece and placed in a cold balanced salt solution (1.45×10^-2 M Tris, 1.26×10^-1 M NaCl, 5×10^-8 M KCl, 10^-3 M MgCl2, 5×10^-6 M CaCl2, and 0.1 percent glucose, pH 7.6). The tissue was removed, blotted, quickly frozen, and stored at...
usually frozen in one tube and was used within 2 weeks, even though receptor binding remained stable for at least 4 months.

Preparation of extract. The eye tissue (1 to 1.5 gm.) was defrosted, finely minced, and homogenized in 2x vol. of Tricine buffer solution (2x10⁻⁴M Tricine, 2x10⁻⁴M CaCl₂, 10⁻³M MgCl₂, 5x10⁻³M dithiothreitol, pH 7.9) in a Brinkmann Polytron (PT 10) homogenizer (two 5 sec. bursts at maximum speed in an ice bath). The homogenate was centrifuged at 100,000 x g for 50 min., and the supernatant was removed and used immediately, since overnight storage at 0° resulted in a loss of 80 percent of the binding activity. The protein concentration in these supernatant extracts was usually 3 to 5 mg/ml.

Incubation with steroids. All nonradioactive steroids* were purchased from Steraloids, Inc., dissolved in absolute ethyl alcohol, and kept at -70° C. The tissue from five or six animals was usually frozen in one tube and was used within 2 weeks, even though receptor binding remained stable for at least 4 months.

Separation of bound and free 1,2-⁹H-dexamethasone. The incubated samples were analyzed by gel filtration with Sephadex G-50* (Pharmacia Fine Chemicals) and eluted with Tricine buffer. Small columns with a void volume of 2.8 ml. were used. Fractions of 0.4 ml. were collected, transferred to scintillation vials containing 10 ml. of Liquifluor in toluene, and counted in an Isocap 300 scintillation counter (⁹H efficiency 43 percent).

Miscellaneous methods. Approximate molecular weight estimates were made by gel filtration with Sephacryl S-200 (Pharmacia). The purity of the labeled dexamethasone, before and after incubation, was checked by appropriate thin-layer chromatography.¹

Results and discussion. Extracts of iris—ciliary body were incubated at 0° with ⁹H-dexamethasone (final concentration, 10⁻³M). A typical elution pattern of gel filtration with Sephadex G-50 after 3 hr. of incubation is shown in Fig. 1 (solid line). About 0.3 percent of the label elutes with the void volume, indicating its binding to a large macromolecular complex. The macromolecular binding of the labeled dexamethasone was significantly (approximately 95 percent) suppressed when a 200-fold excess of nonlabeled steroid was included in the incubation mixture (Fig. 1, dotted line). From this, the specific protein bound radioactivity was calculated (see legend). Experiments of various time courses of incubation demonstrated that 2 hr. at 0° was sufficient to attain maximum binding, which remained constant for at least 24 hr. By contrast, when extracts were warmed to 40° for 30 min., even in the presence of dexamethasone, 90 percent of the specific binding was eliminated, indicating that the binding was to a very heat-labile protein. Furthermore, this specific binding of dexamethasone was not due to any serum contamination, since rabbit sera, diluted to a protein concentration similar to the extracts, revealed only a negligible (<5 percent) amount of specific binding.

Fig. 2, A, shows the amount of ⁹H-dexamethasone bound, with and without an excess of nonlabeled dexamethasone, as a function of dexamethasone concentration. Even at concentrations as low as 5x10⁻⁴M, specific binding was detected. The equilibrium constant for the dissociation of the bound steroid, Kᵣ, calculated from the Scatchard plot of two separate experiments was 1.6 and 2.3x10⁻¹³M, and the amount of dexamethasone bound at saturation was 3.55 and 5.94 x 10⁻¹².

---

*Generic names of steroids: cortisol = 11β,17,21-trihydroxy-9α,11β,21-trihydroxy-17α,20- inseo-20-dione; dexamethasone = 1,4-pregnen-5α,6-20-dione; progesterone = 5α-pregnan-3α,20-dione; triamcinolone = 5α-androstane-17β-ol-3-one; estradiol = 3β-3x(10-estradiol-3β-ol-3-one; progesterone = 5α-pregnan-3α,20-dione; tetrohydrocortisol = 3α,11β,17β,21-tetrahydroxy-5α-pregnan-20-one.
mol/mg of protein. These findings are in agreement with the data obtained in other rabbit dexamethasone target tissues. The linear Scatchard plot is indicative of a single class of binding sites. We have ascertained, using appropriate thin-layer chromatography, that the purity of $^3$H-dexamethasone bound to the specific receptor protein was greater than 95 percent.

Competition experiments with a variety of steroid hormones were undertaken to further characterize the specificity of the dexamethasone binding. The results are seen in Table I. All incubations were carried out for 2 to 3 hr. at 0°. Labeled dexamethasone ($5x10^{-8}M$) was present in all incubates. Various nonlabeled steroids were present at $10^{-6}M$ and $10^{-7}M$, i.e., in 20- and 200-fold molar excess. As can be seen, cortisol is the strongest competitor next to dexamethasone. Progesterone, although not possessing glucocorticoid activity, significantly suppresses dexamethasone binding. The sex steroids, estradiol and dihydrotestosterone, show little competition at $10^{-6}M$ and only partial competition at $10^{-7}M$. The biologically inactive tetrahydrocortisol shows almost no competition even at the higher concentration. This pattern of competition is virtually identical to that obtained with the dexamethasone receptor in the liver.

Receptor molecules are known to be present in aggregate forms at low salt concentrations and in the presence of calcium and magnesium ions. When the $^3$H-dexamethasone-labeled supernatant extract (centrifuged at 100,000 × g) was prepared in the usual manner (see above) and subjected to gel filtration with Sephacryl S-200, the specific binding appeared in the void volume of the column, indicating a large-molecular species (greater than 250,000 daltons). In order to demonstrate the aggregate nature of this receptor, the tissue extract was prepared in Tris buffer solution void of divalent ions (2x10^{-3}M Tris, 2x10^{-3}M EDTA, 5x10^{-4}M dithiothreitol, pH 7.4) and incubated with labeled dexamethasone at 0° for 6 hr. KCl was then added to a final concentration of 0.4M, and an aliquot was again chromatographed on the Sephacryl column. The protein was eluted with 0.4M KCl containing Tris buffer. It can be seen in Fig. 3 that most of the specifically bound steroid penetrates the gel and elutes

---

**Table I. Competition of various steroids with the binding of $^3$H-dexamethasone to the receptor**

<table>
<thead>
<tr>
<th>Nonlabeled steroid</th>
<th>Percent of $^3$H-dexamethasone bound in the presence of nonlabeled steroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrahydrocortisol</td>
<td>88 (20-fold excess)</td>
</tr>
<tr>
<td>Estradiol</td>
<td>95 (20-fold excess)</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>77 (20-fold excess)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>40 (20-fold excess)</td>
</tr>
<tr>
<td>Cortisol</td>
<td>19 (20-fold excess)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>9 (20-fold excess)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>90 (200-fold excess)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>42 (200-fold excess)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>33 (200-fold excess)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>9 (200-fold excess)</td>
</tr>
</tbody>
</table>

$^3$H-Dexamethasone concentration was $5x10^{-8}M$, and incubation was for 2-3 hours at 0°.
Fig. 3. Elution profile in high salt on Sephacryl S-200 of \(^{3}H\)-dexamethasone bound to its receptor. Before chromatography, aliquots were adjusted to 0.4M KCl. The extract was incubated in the presence (interrupted line) and absence (solid line) of a 200-fold excess of nonlabeled dexamethasone.

at a position corresponding to a lower molecular weight (approximately 50,000 daltons) but that some of the specifically bound steroid still remains in the void volume. This “salt shift,” which dissociates the polymeric form of the receptor, is in agreement with the nature of other steroid cytoplasmic receptors. It is also noteworthy that the lower-molecular species is almost completely suppressible in the presence of a 200-fold excess of unlabeled dexamethasone.

The properties of this specific dexamethasone-binding cytosolic protein include (1) a high affinity and low capacity for the steroid, (2) successful competition with biologically active, but not inactive, glucocorticoids or other steroids, (3) extreme heat sensitivity, (4) absence of contamination by serum proteins, and (5) characteristic physiochemical behavior in various salt concentrations. These characteristics of the dexamethasone-binding protein, described in this report, suggest that the rabbit iris-ciliary body contains a specific glucocorticoid receptor.

We have previously shown that the iris-ciliary body is capable of metabolizing a variety of steroid hormones, including glucocorticoids. Thus the demonstration of a specific dexamethasone receptor in these tissues provides the first direct biochemical evidence that the iris-ciliary body may function as a target organ for glucocorticoids. Since the iris-ciliary body consists of various cellular elements, experiments are now in progress to determine the localization of the receptor in one or more of these structures.

We wish to acknowledge the expert and dedicated technical assistance of Mrs. Helena S. Yeh.

From the Department of Medicine, Section of Endocrinology, and the Department of Ophthalmology, New York Medical College, New York, N.Y. These studies were supported by grant EY-01313 of the National Eye Institute, National Institutes of Health, Bethesda, Md. Submitted for publication May 23, 1977. Reprint requests: A. Louis Southren, M.D., Department of Medicine, New York Medical College, Flower & Fifth Avenue Hospitals, 5th Avenue at 106th St., New York, N.Y. 10029.

Key words: glucocorticoid receptor, iris-ciliary body.

REFERENCES


Intraocular IgE antibody induced in guinea pigs with Ascaris suum larvae. JOHN J. DONNELLY, JOHN H. ROCKEY, AND E. J. L. SOULSBY.

The systemic and local (intraocular) IgE antibody responses to infective and heat-killed Ascaris suum...