References and Notes

1. U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367,** 1 (1971).
2. ———, *ibid.*, p. 95; B. R. Cooper, J. L. Howard, L. D. Grant, R. D. Smith, G. R. Breese, *Pharmacol. Biochem. Behav.* **2,** 639 (1974); H. C. Fibiger, A. P. Zis, E. G. McGeer, *Brain Res.* **55,** 135 (1973); J. F. Marshall, J. S. Richardson, P. Teitelbaum, *J. Comp. Physiol. Psychol.* **87,** 808 (1974); G. A. Oltmans and J. A. Harvey, *Physiol. Behav.* **8,** 69 (1972); G. P. Smith, A. J. Strohmayer, D. J. Reis, *Nature (London) New Biol.* **235,** 27 (1972); E. M. Stricker and M. J. Zigmond, *J. Comp. Physiol. Psychol.* **86,** 973 (1974).
3. For example, S. Fahn, *Adv. Neurol.* **14,** 59 (1976).
4. H. Shinozaki and S. Konishi, *Brain Res.* **24,** 368 (1970); R. M. McCullock, G. A. R. Johnston, C. J. A. Game, D. R. Curtis, *Exp. Brain Res.* **21,** 515 (1974).
5. J. T. Coyle and R. Schwarcz, *Nature (London)* **263,** 244 (1976); E. G. McGeer and P. L. McGeer, *ibid.*, p. 517; R. Schwarcz and J. T. Coyle, *Life Sci.* **20,** 431 (1977); *Brain Res.* **127,** 235 (1977). The neurochemical changes seen in animals injected with kainic acid suggest that the drug destroys neurons whose perikarya are near the injection site, but leaves relatively intact those neurons whose axons or terminals pass through this area. However, this hypothesis has not been proven, and the mechanisms underlying kainic acid–induced neural degeneration remain unknown.
6. P. Teitelbaum and A. N. Epstein, *Psychol. Rev.* **69,** 74 (1962); L. D. Lytle, in *Nutrition and Brain*, R. J. Wurtman and J. J. Wurtman, Eds. (Raven, New York, 1977), vol. 1, pp. 1–145.
7. Different groups of animals ($N \geq 10$) were injected with α-methyl-aspartic acid or with 1.0 μg (unilaterally or bilaterally), 2.5, or 5.0 μg (bilaterally) of kainic acid. The stereotaxic coordinates for the injections in the midcaudate nucleus were A-P 8.6 mm and D-V 4.9 mm from the interaural line and M-L 2.7 mm from the midsagittal sinus suture. Some animals were perfused with 10 percent formalin, and 50-μm sections through the caudate nucleus were stained with cresyl violet. Kainic acid caused a marked cell body loss throughout this brain region, with the greatest damage occurring within a radius of 1.5 to 2.0 mm around the injection site.
8. The assay procedures are described as follows. Dopamine: J. T. Coyle and D. Henry, *J. Neurochem.* **21,** 63 (1971); GABA: J. T. Graham and M. H. Aprison, *Anal. Biochem.* **15,** 487 (1966); protein: O. H. Lowry, N. J. Rosenbrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193,** 265 (1951); choline acetyltransferase activity: B. Mannervik and B. Sorbo, *Biochem. Pharmacol.* **19,** 2509 (1970); and glutamate decarboxylase activity: J. R. Moskal and S. Basu, *Anal. Biochem.* **65,** 449 (1975).
9. No special postoperative recovery techniques were used. However, the period of aphagia and adipsia, and subsequent deaths in some groups of kainic acid–treated animals, were not merely the result of nonspecific drug toxicity. In other experiments we have maintained animals given high doses of kainic acid (2.5 or 5.0 μg, bilaterally) by force-feeding them high-calorie liquid diets. These animals eventually recover spontaneous feeding and drinking; however, they continue to weigh less than control animals, and they do not respond normally by increasing water intake following challenges with hypertonic saline injections (D. C. Pettibone, N. Kaufman, L. D. Lytle, in preparation). Rats similarly recovered from bilateral chemical (*2*) or electrical (*6*) lesions of the nigrostriatal bundle show other permanent feeding and drinking regulatory deficits, including impaired responses to glucoprivic or osmotic challenges, finicky responses to changes in diet palatability, and blunted responses to anorexic doses of amphetamine. Although kainic acid–treated animals share some of these deficits, it is too early to tell whether they are identical in all respects to those seen in animals with nigrostriatal lesions.
10. Animals with unilateral intracaudate injections of kainic acid (3.0 μg) show marked contralateral rotational behavior when tested 1 day after injection (33 ± 6 rotations per 15 minutes in kainic acid–treated animals compared to 3 ± 1 rotations in those treated with α-methyl-aspartic acid). This contralateral rotational preference gradually disappears, and is not apparent by 10 days after treatment. However, animals given unilateral injections of kainic acid show a strong ipsilateral rotational preference when challenged with *d*-amphetamine sulfate 10 days after kainic acid injection [25 ± 4 ipsilateral rotations per 15 minutes in kainic acid–treated animals compared to 9 ± 4 rotations in control injected rats ($P < .01$) (D. J. Pettibone, N. Kaufman, L. D. Lytle, in preparation). This drug-induced turning behavior is similar to that seen in animals with unilateral nigrostriatal lesions [U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367,** 49 (1971)].
11. Some evidence indicates that animals treated with doses of kainic acid large enough (> 1.0 μg) to reduce the activities of choline acetyltransferase and glutamate decarboxylase by more than 70 percent do not recover spontaneous eating and drinking unless force-fed with liquid diets.
12. The evidence that striatal cholinergic neurons are intrinsic is convincing [for example, E. G. McGeer, P. L. McGeer, D. S. Grewaal, V. K. Singh, *J. Pharmacol.* **2,** 143 (1975); F. Fonnum, *Brain Res.* **62,** 497 (1973); I. J. Bak, W. B. Choi, R. Hassler, *Adv. Neurol.* **9,** 13 (1975)] whereas support for the hypothesis that these cells form synapses in the caudate nucleus with dopamine nigrostriatal afferent nerves is largely indirect but compelling [P. M. Groves, C. J. Wilson, S. J. Young, G. V. Rebec, *Science* **190,** 522 (1975); V. H. Sethy and M. H. Van Woert, *Nature (London)* **251,** 529 (1974); D. L. Cheny, E. Costa, G. Kacagni, M. Trabucci, *Br. J. Pharmacol.* **52,** 427 (1974); P. G. Guyenet, Y. Agid, F. Javoy, J. C. Beaujouan, J. C. Rossier, J. Glowinski, *Brain Res.* **84,** 227 (1975)]. The evidence that GABA-containing perikarya are localized in the caudate-putamen or the globus pallidus is controversial [for example, P. L. McGeer and E. G. McGeer, in *GABA in Nervous System Function*, E. Roberts, T. N. Chase, D. B. Tower, Eds. (Raven, New York, 1976), pp. 487–495; J. S. Kim, I. J. Bak, R. Hassler, Y. Okada, *Exp. Brain Res.* **14,** 95 (1971)]; however, it is generally agreed that an inhibitory pathway to the substantia nigra is GABAergic [Y. Okada, in *GABA in Nervous System Function*, E. Roberts, T. N. Chase, D. B. Tower, Eds. (Raven, New York, 1976), pp. 235–243; M. Yoshida and W. Precht, *Brain Res.* **32,** 225 (1971); W. Precht and M. Yoshida, *ibid.*, p. 229].
13. J. S. Hong, H.-Y. T. Yang, G. Racagni, E. Costa, *Brain Res.* **122,** 541 (1977).

14. Supported in part by NIMH grant MH-25075 and by an Alfred P. Sloan Foundation fellowship in neurosciences to L.D.L.

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Receptors for Glucocorticoids in the Lens Epithelium of the Calf

Abstract. *The calf lens epithelium contains a specific cytoplasmic receptor for glucocorticoids. This binding protein has a high affinity for dexamethasone (average dissociation constant, 8×10^{-9} mole per liter), a low capacity (average, 550 femtomoles per milligram of protein), extreme heat sensitivity, and exhibits a pattern of competition similar to that of glucocorticoid receptors in other tissues. This provides direct biochemical evidence that these tissues may function as a target organ for glucocorticoids.*

Glucocorticoids have been shown to induce posterior subcapsular cataract in man following both topical and systemic administration (*1*). It is not known, however, whether the steroid hormones produce this effect by a direct action on lens tissue or through some secondary alteration of metabolism at another site. Since the primary action of a steroid hormone is its binding to a cytoplasmic receptor protein, we have initiated a search for glucocorticoid receptors in lens tissue. We report here on the presence of a glucocorticoid receptor in calf lens epithelium, which provides direct biochemical evidence that these cells may function as a target organ for glucocorticoids.

Calf lens was used since large amounts of tissue were readily available (*2*). For each experiment we used extracts prepared from 20 to 50 anterior capsules with adhering epithelial cells (*3*). The

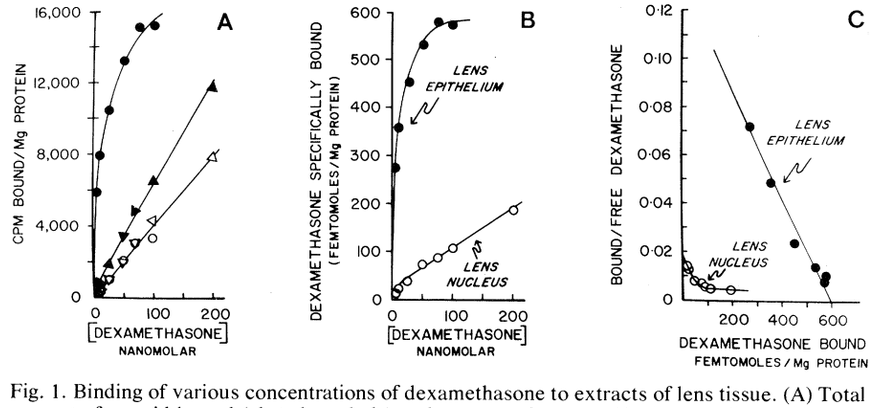

Fig. 1. Binding of various concentrations of dexamethasone to extracts of lens tissue. (A) Total amount of steroid bound (closed symbols) and amount of nonspecifically bound steroid (open symbols) for extracts from the lens epithelium (circles) and the lens nucleus (triangles). (B) Nonspecifically bound steroid has been subtracted from the total, and the specifically bound steroid is expressed in femtomoles per milligram of protein in the extracts. (C) Scatchard plot of the specifically bound dexamethasone. The equilibrium constant for the dissociation of the bound steroid, K_D, calculated from the slope, is $6 \times 10^{-9} M$. The protein concentrations in the extracts were: lens nucleus, 4.7 mg/ml, and lens epithelium, 1.26 mg/ml.

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extracts were incubated with varying concentrations of tritium-labeled dexamethasone (specific activity, 20 Ci/mmole) at 0°C for 18 to 20 hours unless otherwise specified. The incubate was then applied to a small Sephadex G-50 column to separate macromolecule-bound from free hormone, as recently described for iris and ciliary body (*4*).

Figure 1A is a typical experiment showing the amount of [^{3}H]dexamethasone bound, with and without an excess of nonlabeled dexamethasone, to extracts prepared from lens epithelium and from lens nucleus at various hormone concentrations. The nonspecific binding—that is, the amount of radioactivity still bound in the presence of a large excess of nonlabeled steroid—is the same for both of these tissues. The total amount of dexamethasone bound, however, is much greater in the epithelium than in the lens nucleus. Figure 1B indicates the amount of dexamethasone specifically bound to extracts of the lens epithelium and lens nucleus as a function of steroid concentration. At concentrations as low as $5 \times 10^{-9}M$ specific binding of the steroid to the lens epithelium is readily seen, and saturation is reached at a concentration of about $5 \times 10^{-8}M$. By contrast, the specific binding of dexamethasone to extracts of lens nucleus is low and does not approach saturation even at steroid concentrations as high as $2 \times 10^{-7}M$. In the Scatchard plot (Fig. 1C), the high affinity ($K_D = 6 \times 10^{-9}M$) and low capacity (600 femtomoles per milligram of protein) of the steroid binding to the extract from lens epithelium is apparent (*5*). The linear Scatchard plot obtained with lens epithelium indicates a single class of binding sites. On the other hand, dexamethasone binding to the lens nucleus exhibits high capacity with no evident saturation. Although a small amount of high-affinity binding sites may be present in the lens nucleus, the concentration of these sites is at most 10 percent of that found in the lens epithelium. This finding is consistent with the origin of this tissue from lens epithelium.

Figure 2 demonstrates the extreme heat lability of dexamethasone binding to the lens epithelium. At 20° and 30°C significant binding activity is destroyed, indicating that the binding is to a very heat-labile material. By contrast, steroid binding to the lens nucleus is stable even at 50°C.

Competition experiments with a variety of steroid hormones were undertaken to further characterize the specificity of the dexamethasone binding to extracts from lens epithelium (*4*). Cortisol is the strongest competitor after dexamethasone. As expected, progesterone, while not possessing glucocorticoid activity, significantly suppresses dexamethasone binding. The sex steroids estradiol and dihydrotestosterone show virtually no competition, whereas the biologically inactive 20β-cortol and 20β-dihydrocortisol demonstrate no competition when present in a 20-fold molar excess and only slight competition (40 percent) when present in a 200-fold molar excess ($10^{-5}M$).

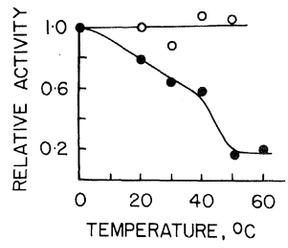

Fig. 2. Thermal stability of specific dexamethasone binding activity of lens tissue. Extracts from the lens epithelium (closed circles) and the lens nucleus (open circles) were heated to the indicated temperatures for 30 minutes, cooled to 0°C, and then incubated with [^{3}H]dexamethasone overnight. The amount of specifically bound dexamethasone is expressed relative to that in an unheated control. The protein concentrations in these extracts and times of incubation were: lens nucleus, 4.6 mg/ml, 17 to 20 hours; and lens epithelium, 0.81 mg/ml, 0.5 to 3 hours.

Since all preparations of lens epithelium contain contaminating capsular material, the posterior capsule, which does not contain an underlying epithelial layer, was assayed as a control. The tissue was found to have only a small amount of steroid-binding activity (less than 10 percent of that of lens epithelium), which was largely heat-stable. Similarly, aqueous humor showed only negligible steroid-binding activity. Thus, the high amount of heat-labile steroid-binding activity seen in the extracts of lens epithelial cells is not due to contaminating capsular material or aqueous humor. In addition, an extract of the lens epithelium was incubated with labeled dexamethasone for 21 hours at 0°C to determine whether dexamethasone or a minor contaminant was actually binding to the receptor. The bound steroid was isolated, extracted with ethyl acetate, and analyzed by thin-layer chromatography as described previously (*6*). More than 90 percent of the bound material was found to migrate as authentic dexamethasone.

The presence of high-affinity and low-capacity binding, the extreme heat sensitivity, and the pattern of competition all suggest that the lens epithelium contains a specific cytoplasmic glucocorticoid receptor both qualitatively and quantitatively similar to that found in other target tissues such as the liver (*7*). The normal pattern of differentiation of lens epithelial cells into mature fiber cells is accompanied by characteristic morphological and biochemical events. Among these are the synthesis of the messenger RNA's coding for the crystallins and a change in the pattern of lactate dehydrogenase isoenzymes (*8*). In view of our finding of glucocorticoid receptors in lens epithelial cells, these steroids may have a physiological role in the regulation of differentiation of these cells. Further studies are necessary to determine the mechanism by which glucocorticoids affect cell differentiation and, under pharmacological conditions, produce a posterior subcapsular cataract.

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References and Notes

1. B. Becker, *Am. J. Ophthalmol.* **58**, 872 (1964); E. Frandsen, *Acta Ophthalmol.* **42**, 108 (1964); C. L. Giles, G. L. Mason, I. F. Duff, J. A. McLean, *J. Am. Med. Assoc.* **182**, 719 (1962).
2. Eyes were obtained immediately after slaughter and were kept cold until dissection, usually within 3 hours. The cornea and iris were removed, exposing the lens in situ. To recover significant numbers of subcapsular epithelial cells without contamination by cortical lens fibers, a small radial incision was made at the equator of the lens in situ and the anterior capsule with the adhering epithelial cells was teased off the lens. The nuclear region of the lens (lens nucleus) was obtained by removing all of the loose cortical fibers. To obtain the posterior capsule (which does not contain adhering epithelial cells) a separate series of eyes was used. The eyes were opened posteriorly, the vitreous was scraped away exposing the posterior surface of the lens, and the posterior capsule was removed as described for the anterior capsule. All tissues were frozen immediately at −80°C and assayed within 3 weeks.
3. The tissue was suspended in 2 ml of Tricine buffer solution ($2 \times 10^{-2}M$ Tricine, $2 \times 10^{-3}M$ $CaCl_2$, $10^{-3}M$ $MgCl_2$, and $5 \times 10^{-4}M$ dithiothreitol, *p*H 7.9), homogenized with a Brinkman Polytron (PT 10-ST) homogenizer at low speed, and centrifuged at 100,000*g* for 50 minutes to obtain a particle-free cytosol. Protein concentrations in the cytosol, determined by the Lowry method, were usually in the range 1 to 5 mg/ml.
4. B. I. Weinstein, K. Altman, G. G. Gordon, M. Dunn, A. L. Southren, *Invest. Ophthalmol.* **16**, 973 (1977).
5. Duplicate experiments with extracts from lens epithelium have given an average K_D of $8 \times 10^{-9}M$ and an average capacity of 550 fmole/mg.
6. A. L. Southren, K. Altman, J. Vittek, V. Boniuk, G. G. Gordon, *Invest. Ophthalmol.* **15**, 222 (1976).
7. J. D. Baxter and G. M. Tomkins, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 932 (1971); P. L. Ballard *et al.*, *Endocrinology* **94**, 998 (1974).
8. A. Spector and D. Travis, *J. Biol. Chem.* **241**, 1290 (1966); J. A. Stewart and J. Papaconstantinou, *J. Mol. Biol.* **29**, 357 (1967); J. Papaconstantinou, *Science* **156**, 338 (1967); P. Zelenka and J. Piatigorsky, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1896 (1974).
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