Glucocorticoid localization by radioautography in the rabbit eye following systemic administration of ^{3}H-dexamethasone[1]

A. N. Tchernitchin and Nina Tchernitchin

Laboratory of Experimental Endocrinology, Department of Experimental Morphology, University of Chile Medical School, Casilla 21104, Correo 21, Santiago (Chile), and Departments of Anatomy, Ophthalmology and Medicine, New York Medical College, Valhalla (N.Y. 10595, USA), 11 February 1980

Summary. Dexamethasone was localized radioautographically in the nuclei of target cells of the rabbit eye, following i.v. administration of the labeled steroid. Nuclear receptors in stromal and endothelial cells of the outflow pathway region suggest that glucocorticoids may alter the outflow facility by specific responses of target cells.

Glucocorticoid administration has been shown to increase intra-ocular pressure in rabbits and humans[2,3]. The presence of a dexamethasone-binding protein, exhibiting many of the biochemical properties of a glucocorticoid receptor in homogenates of rabbit iris, ciliary body and adjacent corneal scleral tissue has been previously demonstrated[4]. These biochemical methods, however, are not able to identify which cells are the target cells. The identification of glucocorticoid target cells is important since they may be involved in the regulation of intraocular pressure. The localization of receptors in stromal and endothelial cells of the corneoscleral lumbus which interact with aqueous outflow would suggest a mechanism of glucocorticoid action different from that suggested by the localization of receptors in ciliary epithelial cells involved in the production of aqueous humor. In a preliminary report, using a dry radioautographic technique, we have described ^{3}H-dexamethasone localization in target cells of rabbit eye tissues following incubation with the labeled steroid either in vitro or following injection into the anterior chamber[5]. Systemic administration of the labeled steroid, however, provides a wider distribution to all eye tissues, while avoiding any local trauma, and thus offers a more accurate assessment of the distribution of the hormone. We now report on the radioautographic localization of ^{3}H-dexamethasone in rabbit eye tissues following i.v. injection of the labeled steroid.

Material and methods. A radioautographic technique, previously shown to be suitable for the localization of diffusible substances, including steroids, was used for this study[6,7]. Female albino New Zealand rabbits (2 kg) were injected with 1.5–5 mCi of 6,7-^{3}H dexamethasone (26.4 Ci/mM) in saline into the marginal ear vein. The eyes were removed from anesthetized animals at intervals from 5 to 150 min. Small tissue samples were obtained by dissection, 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 cryopump (Thermovac Industr. Inc., Copiague, N.Y.) at −35 °C with a prevacuum produced by an oil diffusion pump backed by a mechanical pump. Freeze-dried sections were pressed on to glass slides which had previously been coated with radioautographic emulsion (NTB-3 Kodak) that had been allowed to dry. After 1–6 months of exposure, radioautographs were developed for 35–75 sec in D-19 developer at 20 °C, fixed and then stained in hematoxylin-eosin or methyl green-pyronine.

Results. Radioautographs demonstrated label predominantly over the nuclei of stromal cells in the iris, ciliary body and outflow pathway region (fig. 1a). In addition, label was demonstrated over the nuclei of endothelial cells of small blood and outflow vessels of the same regions (fig. 1b) and sphincter smooth muscle cells of the iris. Both epithelial cell layers of the iris and ciliary body were labeled, but predominantly in the cytoplasm, not in the nuclei (fig. 2). Nuclear concentration was noted only at the tips of very few ciliary processes. There was also increased concentration of radioactivity in the extracellular space of the stroma of ciliary body processes and in the lumen of epithelial invaginations in both iris and ciliary body (fig. 2). Nuclear concentration of label was also seen in connective tissue cells throughout the choroid. In conjunctiva the epithelial cell nuclei were heavily labeled; stromal cells and endothelial cell nuclei were also labeled (fig. 3).

No nuclear localization was found in the cells of the cornea including epithelial, stromal or endothelial cells. No localization was found in the nuclei of the anterior epithelial cells or bow region cells of the lens.

Discussion. Our study demonstrates localization of ^{3}H-dexamethasone in the nuclei of certain cell types in the rabbit eye. Nuclear localization most probably reflects the existence of a cytosol-nuclear receptor system for glucocorticoids, which mediates the genomic effect of the hormone[8]. It is not possible, however, to exclude the existence of cAMP-mediated[9] or strictly non-genomic mechanisms[10] of steroid hormone action.

The localization of glucocorticoid receptors in specific cells of the outflow pathway region suggests that reduction in

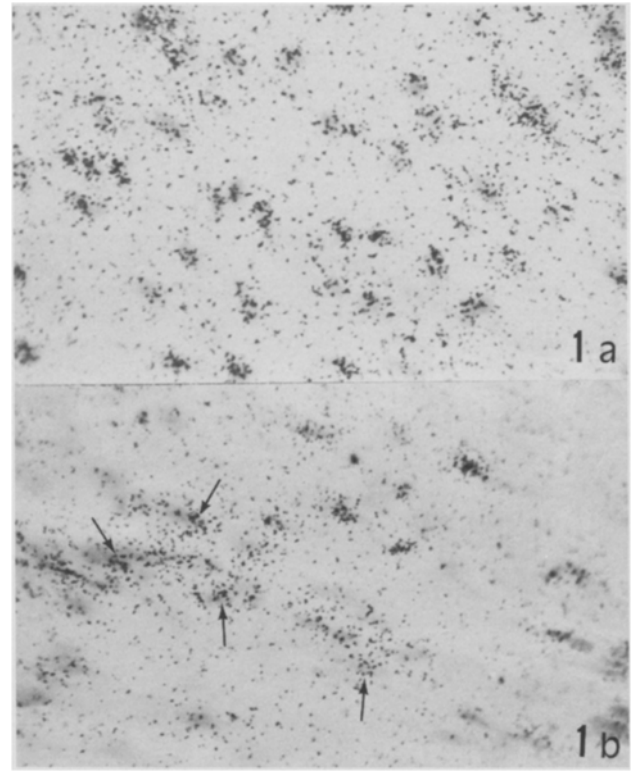

Figure 1. Radioautographs of the outflow pathway region. × 500. This region corresponds to the loose connective tissue at the iridocorneal angle between the sclera and ciliary body. It is homologous to the trabecular meshwork of humans. *a* Nuclear localization in stromal cells. *b* Nuclear localization in endothelial cells of the outflow vessels (arrows).

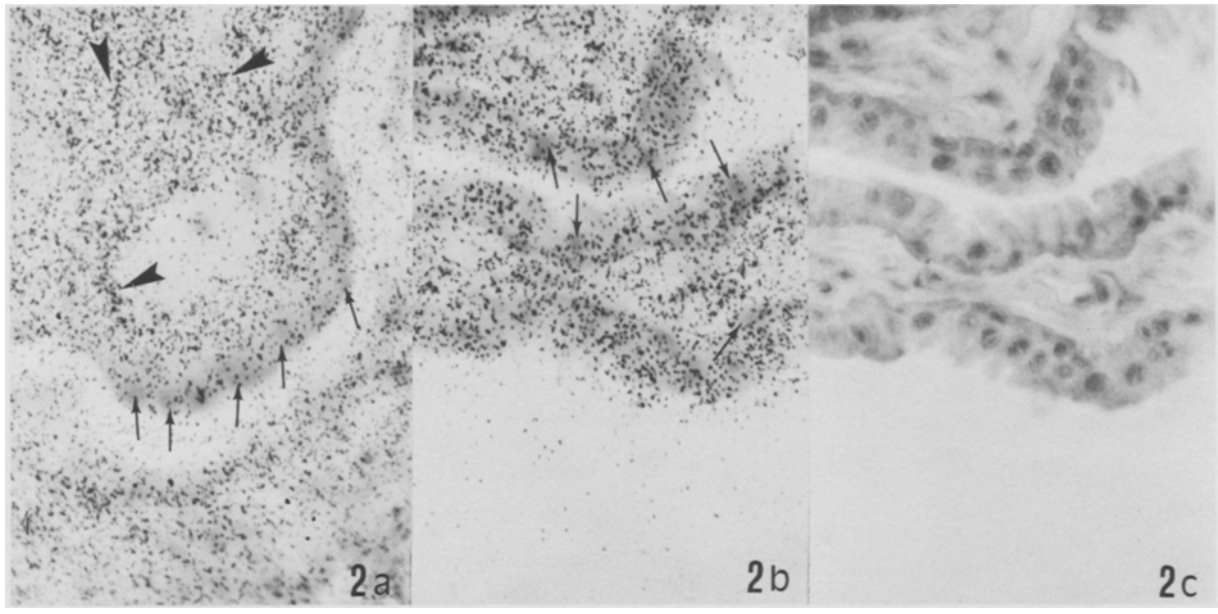

Figure 2. Radioautographs of ciliary processes. × 500. *a* Nuclear exclusion of grains in epithelial cells (arrows). The highest concentration of label is in the extracellular space of the stroma (arrowhead).
b High cytoplasmic concentration of radioactivity in epithelial cells, with nuclear exclusion (arrows). *c* Same field as *b* above, but focused at level of tissue.

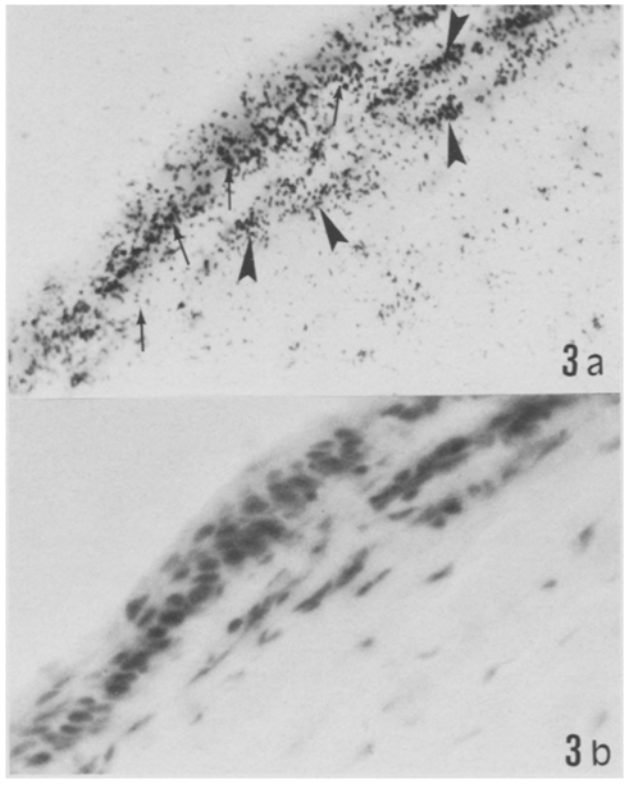

Figure 3. Radioautograph of conjunctiva. ×500. *a* Concentration of radioactivity over basal epithelial layers (arrows) and cells of vessels in conjunctival stroma (arrowheads). *b* Same field as *a* above, but focused at level of tissue.

outflow facility produced by glucocorticoids may be, at least in part, the result of target cell responses that alter the outflow environment. A target cell response that would lead to changes in glycosaminoglycan distribution in the outflow pathway, such as those reported in rabbits treated with dexamethasone[2] could reduce outflow facility.

The lack of nuclear localization in epithelial cells of ciliary body and iris, with the exception of the tips of a few ciliary processes, indicates that this site of aqueous humor production is not a major target tissue for the specific genomic response to dexamethasone.

The finding of glucocorticoid receptors in sphincter smooth muscle cells of the iris, if confirmed in the human, might account for the mydriasis seen after topical steroid use.

The localization of glucocorticoids in the nuclei of epithelial, stromal and vascular endothelial cells of the conjunctiva suggests that these steroids may act in conjunctival inflammation through a specific hormone mechanism. These results do not exclude the existence of other mechanisms for steroid effects on conjunctival inflammation. It is of interest that virtually no nuclear localization of receptors was seen anywhere in the cornea.

Further investigation is necessary to discover the specific responses of each cell-type in the eye which has been found to contain specific receptors to glucocorticoids.

1 Acknowledgments. This work was supported by grant EY-01313 of the National Eye Institute, National Institutes of Health, Bethesda, Md, USA.

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