Autoradiographic Localization of Beta-Adrenergic Receptors in Rabbit Eye

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Using an in vitro autoradiography, beta-adrenergic receptors were localized in the rabbit eye. Autoradiograms were generated by apposition of isotope-sensitive film to slides mounted eye sections, labelled with \( ^{125}\text{I} \) (-) Iodo-cyanopindolol. A high density of silver grains was obtained in conjunctival, corneal and ciliary process epithelium. Binding sites were also present in corneal endothelium, iris epithelium, lens epithelium, choroid and extraocular muscles. In some areas, retina was also labelled. Studies with beta-adrenergic compounds showed that the majority of beta-adrenergic receptors, detectable by autoradiography, were of the beta\(_2\) type in the rabbit eye. Invest Ophthalmol Vis Sci 28:1436–1441, 1987

The presence of the adrenergic neurohumoral system in the eye has been demonstrated in numerous reports. The anatomical, physiological and pharmacological evidence accumulated provides strong support for a rich adrenergic innervation in the uvea and in the ciliary processes and for a high density of adrenergic receptors in the cornea and in the ciliary processes. These reports pointed out that the beta-adrenergic system was predominant in several species, and the clinical value of beta-adrenergic compounds (timolol, metipranolol) in the treatment of glaucoma supported these data. However, probably due to the complexity and diversity of ocular tissues, it is difficult to obtain homogeneous cell types for pharmacological and biochemical studies. This might explain some of the misunderstood and paradoxical aspects of the actions of the adrenergic compounds.

In this report we attempted to localize and characterize beta-adrenergic receptors in whole and undamaged sections of rabbit eye by means of an in vitro autoradiographic technique using \( ^{125}\text{I} \) (-) Iodo-cyanopindolol \( ^{125}\text{ICYP} \) as radioligand.

**Materials and Methods.** Albino and pigmented rabbits were sacrificed by injection of 2 ml Euthanasia 661 (Hoecsht, Somerville, NJ) into the marginal ear vein. Eyes were removed, immersed in Tissue Tek medium, frozen in isopentane cooled (−40°C) in liquid nitrogen, and stored at −80°C. Just before sectioning, the eyes were brought to −20°C and the sections (15 \( \mu \text{m} \) thick) were cut with a cryostat (Bright Instrument, Huntingdon, England), mounted onto gelatin-coated glass slides and stored at −20°C until use. Prior to incubations, sections were allowed to thaw at room temperature. The slides were then incubated in 50 mM Tris HCl, pH 7.5, containing 10 mM MgCl\(_2\), 10 \( \mu \text{M} \) phenylmethylsulfonyl fluoride, 0.02% ascorbate, for 60 min at 20°C. Sections were labelled for autoradiography using a concentration of 20 pM \( ^{125}\text{ICYP} \). Non-specific binding was estimated on adjacent sections in which the \( ^{125}\text{ICYP} \) incubation was carried out in the presence of 1 \( \mu \text{M} \) 1-propranolol. After incubation, the sections were rinsed at room temperature for 20 sec, followed by a 30 min wash in the incubation buffer, and followed by a 20 sec wash in distilled water. The slides were then dried by blowing cold air over the sections, and were placed in X-ray cassettes and apposed to \[^{3}\text{H}\] Ulrofilm (LKB, Bromma, Sweden). After a 5- to 7-day exposure at room temperature, the film was developed in Kodak D-19 for 4 min at 20°C, fixed and dried. The sections were subsequently stained in a trichromic solution (hematoxylin, phloxine, saffron). In displacement studies, we used the following drugs at a concentration of 1 \( \mu \text{M} \): timolol, epinephrine (Sigma Chemical Co., St. Louis, MO), IPS 339 (Prof. Leclerc Strasbourg), desacetyl-metipranolol, metipranolol (Laboratoires Dulcis, Monaco), atenolol, practolol (ICI Pharma, Enghien-Les-Bains, France), phenolamine (CIBA GEIGY, Rueil-Malmaison, France). All the data presented here were reproducible and obtained in several sections from the same or three different animals. These investigations conformed to the ARVO Resolution on the Use of Animals in Research.
**Results.** Preliminary studies have indicated that the incubation and washing conditions gave an optimal, reproducible labelling of beta-adrenergic receptors; these conditions provided a maximal specific-to-non-specific ratio as shown in Figure 1. The data revealed the localization of beta-adrenergic receptors on whole eye sections and the difference in the labelling between albino and pigmented eye. In the anterior segment of the eye, conjunctiva, cornea and ciliary processes were intensively labelled. Choroid, lens, retina and extraocular muscles showed also some binding sites.
There were no differences in the labelling between the non-pigmented structures in the pigmented eye and the equivalent tissues in the albino eye. Since the ocular pigment, melanin, was known to bind intensively and non-specifically to numerous substances and particularly beta-adrenergic compounds (Fig. 1, panel 4), it was impossible to determine if a specific binding in the pigmented structures (ciliary process, iris, retinal pigmented epithelium, choroid) was present in the pigmented eye.

Microscopic analysis of autoradiograms and stained sections further localized beta-adrenergic re-
ceptors in structures of albino rabbit eye. In the conjunctiva, the epithelium was heavily labelled (not shown). In the cornea (Fig. 2, panels 1, 2), the epithelium and the endothelium showed a high density of silver grains; the stroma was slightly labelled. $^{125}$I-CYP binding sites were also found in the ciliary epithelium, but not in the stroma (Fig. 2, panels 3, 4). Epithelium of the iris showed beta-adrenergic receptors (Fig. 2, panels 5, 6). It was also evident that the lens epithelium was labelled (Fig. 3, panels 1, 2). Few
In order to characterize these receptors, we have experimented $^{125}\text{I}}$CYP binding in the presence of several adrenergic compounds ($10^{-6}$ M) (Fig. 4). In the albino rabbit eye, when timolol, desacetyl-metipranolol (main metabolite of metipranolol), or IPS 339 (beta$_2$-selective) were incubated with $^{125}\text{I}}$CYP, no labelling was observed on sections. Incubation with metipranolol or epinephrine led to a slight labelling. On the other hand, there were no differences between incubation with $^{125}\text{I}}$CYP alone or plus atenolol, practolol (beta$_1$-selective) or phentolamine (alpha antagonist). In the pigmented rabbit eye, the same results were obtained in the non-pigmented structures. The order of potency of these compounds in inhibiting $^{125}\text{I}}$CYP binding indicated that the majority of sites labelled corresponded to beta$_2$-adrenergic receptor binding sites, on all structures of the rabbit eye.

**Discussion.** Beta-adrenergic binding studies, adenylate cyclase experiments and histological determination of adrenergic innervation have been previously described in these eye structures: ciliary process, iris, choroid and extraocular muscles. The presence of beta-adrenergic receptors has also been established in the cornea and proposed to exist in the lens and in the retina as well. One important function modulated by the adrenergic system is the secretion and the excretion of aqueous humor in the anterior chamber.

In this report, we used a recent technique, in vitro autoradiography, to localize membrane receptors while maintaining the anatomical integrity of the tissue. This technique has been intensively used to localize different binding sites in the brain, and we have extended this approach to macroscopically map beta-adrenergic receptors within the albino and pigmented rabbit eye.

The epithelium of the conjunctiva, the cornea and the ciliary process were heavily labelled with $^{125}\text{I}}$CYP. Corneal endothelium, iris epithelium, lens epithelium, retina, choroid and extraocular muscles also showed binding sites. However, the macroscopic resolving power of this technique did not allow us to: (1) determine whether or not beta-adrenergic receptors were present in the trabeculum area; and (2) map these receptors more precisely in the ciliary epithelium, the retina, the choroid and/or the retinal pigmented epithelium. Some authors, using another autoradiographic technique, have already demonstrated the presence of these receptors in the trabeculum and in the non-pigmented cells of the ciliary epithelium. Pharmacological characterization indicated that the majority of beta-adrenergic receptors (detectable by autoradiography) in the rabbit eye were of a beta$_2$ type and confirmed previous findings in cor-
Nevertheless, the presence of beta₁-adrenergic receptors cannot be ruled out, because: (1) a number of tissues appear to have a mixture of beta₁ and beta₂ subtypes; and (2) IPS 339 (beta₂-selective) is more potent in blocking beta₂ receptors than atenolol or practolol (beta₁-selective) are in blocking beta₁ receptors.

Finally, this study showed that the beta-adrenergic receptors (mainly beta₂) were widely distributed in the rabbit ocular tissues.

**Key words:** autoradiography, beta-adrenergic receptors, eye, rabbit

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