Localization of beta receptors in the anterior segment of the rat eye by a fluorescent analogue of propranolol

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A fluorescent analogue of propranolol, 9-AAP, was injected intravenously in order to detect beta-adrenergic receptors in the anterior segment of the albino rat eye. Specific 9-AAP fluorescence was noted along cell membranes of the ciliary epithelium and to a lesser extent in the walls of blood vessels in the ciliary processes and episclera at the limbus. The iris showed maximum 9-AAP binding in the region of the sphincter muscle. These data suggest that 9-AAP may label beta receptors in the anterior segment of the rat eye.

Key words: 9-amino-acridin-propranolol, fluorescent beta blocker, beta receptors, anterior segment

Catecholamines (CA) such as noradrenaline have been shown to be present in the iris, ciliary body, and the aqueous drainage channels of various animal species.[1] It is known that the effect of CA on postsynaptic membranes is mediated in part by beta-adrenergic receptors. The latter have been shown to be present in the ciliary body, iris, and possibly in the episcleral drainage channels by indirect physiological and pharmacological methods.[2–5]

We have recently developed a direct in vivo histochemical method for the detection of beta receptors in various tissues by the use of 9-amino-acridin-propranolol (9-AAP).[6–8] This potent fluorescent beta-adrenergic blocker was used in the present study in an attempt to detect beta-adrenergic receptors in the anterior segment of the rat eye.

Methods

9-AAP, a fluorescent analogue of propranolol (N-[-2-hydroxy-3-naphthoxy propyl]-N-[-9-amino acridin] isopropyl diamine) was used in this study. It has been known to inhibit competitively the *l*-epinephrine–dependent adenylate cyclase activity in turkey erythrocyte membranes, without affecting the fluoride-stimulated adenylate cyclase activity. In addition, 9-AAP inhibits in a competitive and stereospecific manner the binding of (^{125}I)-hydroxybenzylpindolol to these beta-adrenergic receptors.[9] 9-AAP, when injected into rats, has been shown previously to label specifically beta-adrenergic receptors in the rat central nervous system as well as in other organs.

9-AAP in saline, 2.5 mg/100 gm body weight, was injected into the tail vein of 27 albino rats (200 to 220 gm). Controls included (1) five noninjected animals, to rule out autofluorescence, (2) two ani-

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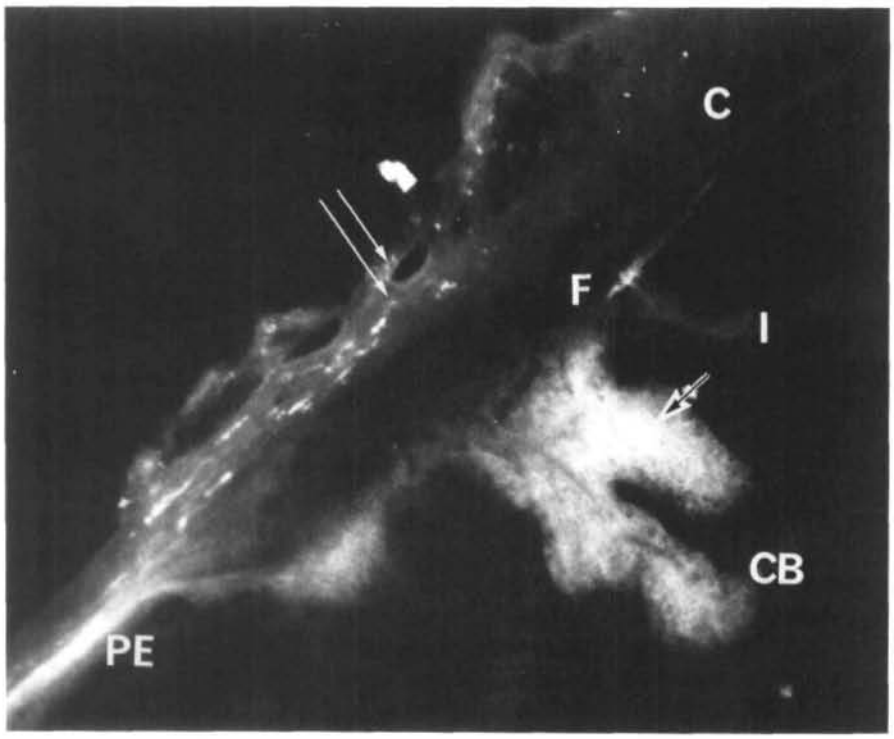

Fig. 1. Fluorescent photomicrograph of the ciliary body *(CB)* and limbal regions of rat treated by 9-AAP. Note fluorescence in the episcleral vessels (two arrows) and ciliary body (one arrow). The filtration region *(F)* is devoid of fluorescence except for a striplike artefact which lies internal to Descemet's membrane. Nonfluorescent cornea *(C)* and *(I)* and autofluorescent retinal pigment epithelium *(PE)* are seen. (Frozen section; ×120.)

mals treated by the fluorescent dye 9-amino-acridin alone, and (3) groups of five animals pretreated with one of the following compounds: (±)-propranolol, (−)-propranolol, or (+)-propranolol (5 mg/100 gm in saline by slow intravenous injection). 9-AAP (2.5 mg/100 gm) was administered to each of the pretreated animals 30 min later. All the animals were killed by decapitation under light ether anesthesia 30 min after injection of 9-AAP. The eyes of all animals were quickly removed and frozen in liquid nitrogen. Later, 10 μ pupil–optic nerve sections were cut on a cryostat at −20°. The frozen sections were mounted on glass slides and air-dried. Sodium phosphate buffer 0.08M, pH 7.5, was applied, and coverslips were placed in position. The sections were observed under phase-contrast and transmitted ultraviolet illumination on a Zeiss Universal fluorescent microscope with HBO 200-W superpressure mercury lamp, exciter filter BG-12, and barrier filter 53. Alternate sections were formalin-fixed, stained by hematoxylin and eosin, and observed under the light microscope.

Results

Accumulations of fluorescent 9-AAP binding sites were seen in the ciliary body, iris, and episcleral tissue at the limbus (Fig. 1). Almost no fluorescence was noted at the ciliary body base in a region corresponding to the ciliary muscle. Few fluorescent dots were present in the stroma of the iris processes, most probably on endothelial cells of the ciliary vessels (Fig. 2, *A* and *B*). The ciliary epithelium showed diffuse membrane fluorescence. The resolution of the sections did not permit localization of the fluorescence to one of the two epithelial layers of the ciliary body. However, it was our impression that the fluorescence was more extensive at the outer ciliary epithelial layer, which corresponds to the pigmented ciliary epithelium (Fig. 2, *A* and *B*). In the iris, diffuse fluorescence could be seen exclusively in the region of the sphincter muscle (Fig. 3). Fluores-

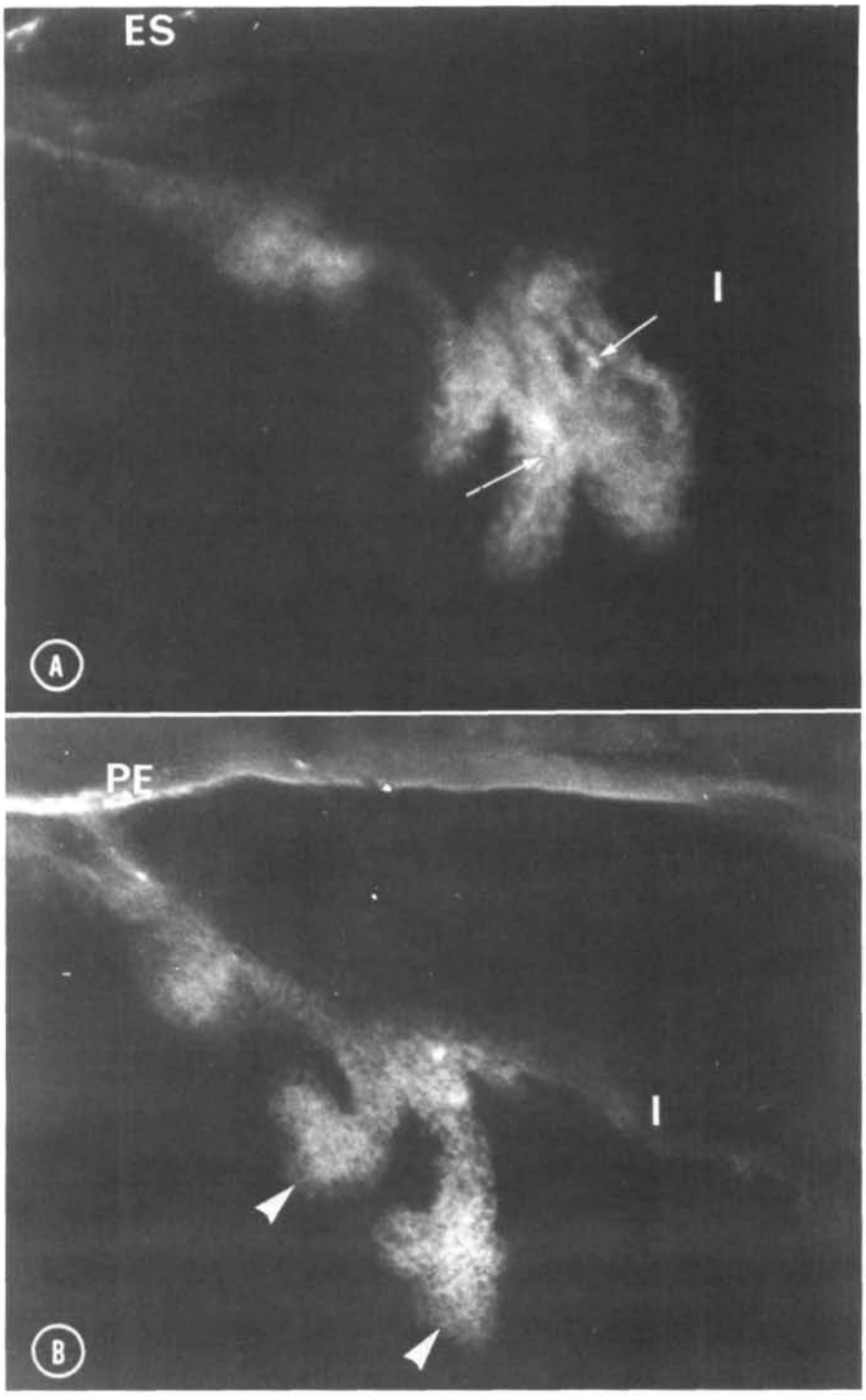

Fig. 2. A, Ciliary body after injection with 9-AAP. Note diffuse fluorescence of the epithelial cells. Few fluorescent dots which may correspond to regions of stromal blood vessels are seen (arrows). The iris base *(I)* is devoid of fluorescence. Fluorescent dots are seen in the episclera *(ES)* as well. (Frozen section; ×160.) **B,** Section through the ciliary body of another animal treated by 9-AAP. The arrows indicate fainter fluorescence on the cell membranes of the inner epithelial layer. The termination of the autofluorescent pigment epithelial layer is seen *(PE)*. (Frozen section; ×120.)

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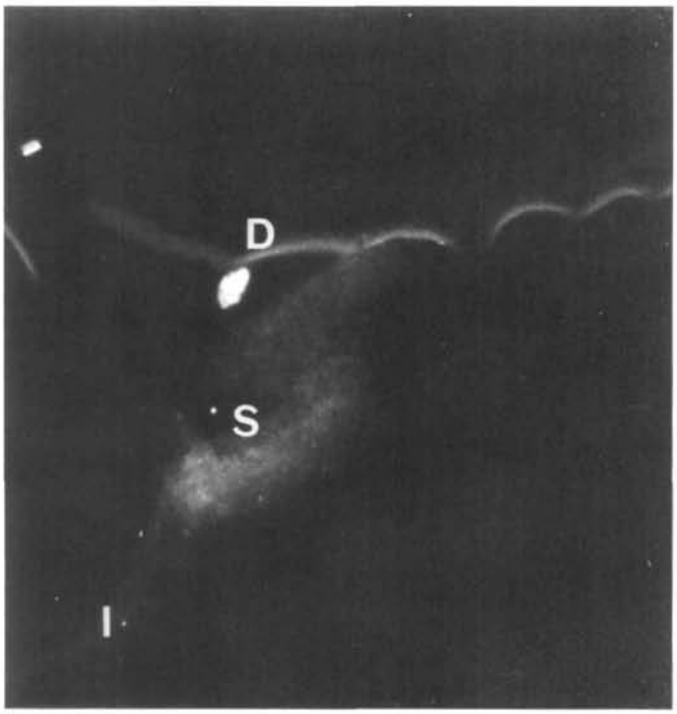

Fig. 3. Folded sphincter region of the iris *(S)* is seen in two focal planes in apposition to Descemet's membrane *(D)*. Note diffuse fluorescence in the sphincter region in contrast to other regions of the iris *(I)*. (Frozen section; ×160.)

Fig. 4. Normal ciliary body *(CB)* and iris *(I)* of a rat. (Frozen section; hematoxylin-eosin stain; ×16.)

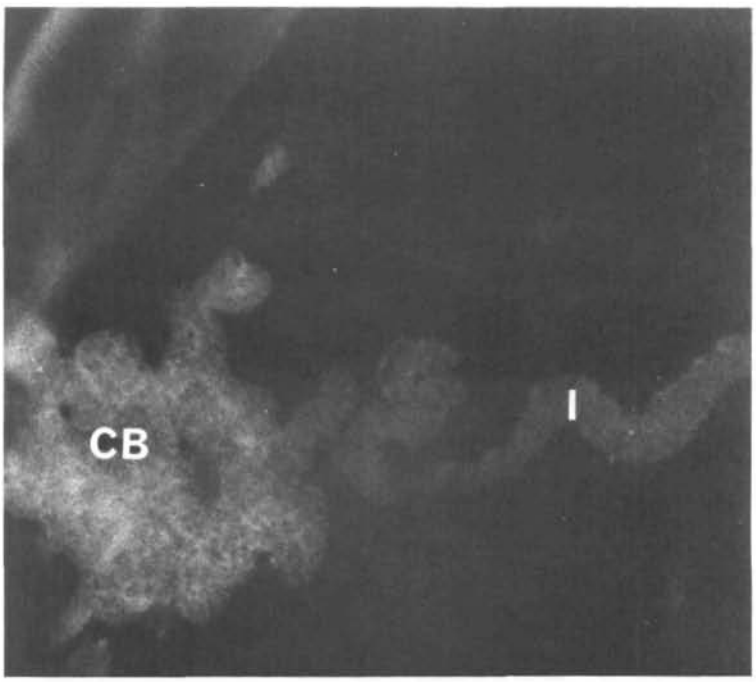

Fig. 5. Ciliary body *(CB)* and iris *(I)* of a rat pretreated by (±)-propranolol. Except for mild haze over the ciliary body, no specific 9-AAP fluorescence is seen. (Frozen section; ×40.)

cence was noted in the walls of blood vessels in the episcleral tissue over the ciliary body (Figs. 1 and 2, A). The filtration area was devoid of fluorescence in most of the sections.

Autofluorescent cells were rarely seen in untreated control animals. When present, they could be differentiated from the 9-AAP labeled fluorescent cells, by their orange fluorescence. Fluorescence was practically absent in sections from control animals treated by 9-amino-acridin alone or pretreated with (±)-propranolol and (−)-propranolol (Fig. 5). In sections from control animals pretreated with (+)-propranolol, the distribution pattern of fluorescence was similar to that observed in animals which were treated by 9-AAP alone, though reduced in intensity.

Discussion

The binding of 9-AAP to beta-receptor sites in the rat eye seems to be a specific one for several reasons. This substance exhibits marked affinity for beta-adrenergic receptors when studied in vitro in a beta-receptor–dependent adenylate cyclase system.

Similarly, it inhibits in a competitive manner the binding of (^{125}I)-hydroxybenzylpindolol to these beta receptors.[9] In addition, the appearance of the fluorescence in vivo can be blocked in a stereospecific fashion by prior injection of the biologically active beta antagonists (±) or (−)-propranolol but not with (+)-.[6] Furthermore, the binding of 9-AAP in other organs such as the cerebellum, cerebral cortex, heart, and kidney is distinctly localized to areas where the presence of beta receptors was indicated by other indirect methods.[6–8]

Studies in experimental animals which used histochemical fluorescence techniques demonstrated extensive networks of presynaptic noradrenaline-containing fibers in the episcleral tissue, trabecular meshwork, ciliary body vessels, and iris.[10–15] In addition, electron microscopic studies have shown dense-cored vesicles in synaptic terminals within these regions.[16–22] In the present study, 9-AAP binding sites were observed in the ciliary epithelium, iris sphincter, episcleral vessels, and some of the ciliary vessels of the rat. We suggest that the latter may

correspond to beta receptor sites in the anterior segment of the rat eye.

In the iris, most of the adrenergic fibers are seen in the dilator area; however, these are present as well in the region of the sphincter muscle.[10–12] Complementary beta-receptor sites were identified in this region and are probably related to the inhibitory effect of beta-active substances on this muscle.[2] In the ciliary body few adrenergic fibers were reported to be present in the muscle fibers.[10–13] Most of the adrenergic fibers are present as adrenergic sheaths around blood vessels. Adrenergic varicosities were observed in the blood vessels of the ciliary processes and under the ciliary epithelium. These fibers were never noted to cross the epithelial basement membrane.[10–15] The presynaptic distribution of CA within the ciliary body of various species parallels the distribution of postsynaptic beta-receptor sites labeled by 9-AAP. These were seen along the cell membranes of the ciliary epithelium and to some extent in the blood vessels of the ciliary processes but were almost nonexistent in the ciliary muscle. Therefore the findings in the present study seem to be complementary to the information which is available concerning the distribution of CA in the anterior segment of the eye.

Extensive pharmacological and physiological experiments indicate that adrenergic drugs have a complex effect on the eye, with considerable species variations. It is generally agreed, however, that both alpha and beta receptors are involved in the regulation of the intraocular pressure. For instance, the net effect of epinephrine, a substance with both alpha and beta activities, is reduction of the intraocular pressure.[23] The mechanism of its action probably varies with the species of mammals which were investigated. Adrenergic agonists may act on various sites in the anterior segment of the eye, such as on the ciliary vessels, the secretory ciliary epithelium, the trabecular meshwork, and the aqueous drainage channels.[24–30] In the rat, 9-AAP binding sites were observed on the ciliary epithelium, the episcleral vessels, and probably on blood vessels of the ciliary processes. We could not, however, identify fluorescent sites in the filtration area. Although pharmacological studies on the rat are not available, the localization of 9-AAP binding sites in these regions seems to support previous results in other species, which claim that beta-adrenergic agonists act mainly on the secretion of aqueous humor and to a lesser extent on the outflow mechanism.[22, 24–28] The distribution of beta receptors in at least three locations in the anterior segment of the rat's eye may provide a partial explanation for the multifaceted effects of beta agonists and beta antagonists[31–33] on the intraocular pressure. Further use of 9-AAP in other species may yield additional information on the CA–beta receptor system in the eye.

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