Beta adrenergic receptors in pigmented ciliary processes

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SUMMARY Beta adrenergic receptors from membrane fragments of pigmented sheep eyes were studied and characterised by ligand binding techniques after the removal of melanin. In a representative experiment the β max (total number of beta receptors) was 394.9 fmol/mg protein. The receptor affinity (K_a) was 440 pM. The potency series of drugs to displace ¹²⁵I-HYP from the receptors was timolol=(−) propranolol>(+)propranolol>salbutamol>practolol. β_1 Receptors were not detected in the ciliary processes. β_2 Receptors were the prominent adrenergic receptors present. The theory as to how beta blockers work in glaucoma, their site of action, and the potential role of β_2 blockers for use in intraocular pressure control is discussed.

Beta adrenergic blockers are competitive inhibitors of catecholamines binding at β -adrenergic receptor sites.¹ Various beta blockers have been shown to reduce intraocular pressure in normal human eyes, glaucomatous patients,²⁻⁷ and in animal eyes.^{8,9} It is presumed that these agents exert their intraocular pressure lowering effect by binding to β -adrenergic receptors in the ciliary processes,¹⁰ but this is controversial.⁸

A major advance in the study of β -adrenergic receptors involves the use of radio-ligand techniques.¹² These allow the identification of the location, number, and subtypes (i.e. β_1 or β_2) of adrenergic receptors.¹³ The presence of melanin in the pigmented layer of the ciliary processes has until recently prevented the application of ligand binding techniques to this tissue, because melanin interferes with the assays. We have recently described a method which overcomes this problem.^{14,15}

The present study demonstrates the presence of β -receptors in membrane fractions obtained from pigmented ciliary processes and characterises these receptors into their constituent subtypes, namely, β_1 or β_2 . The results provide an insight into the mechanism of action of beta blockers currently used in the management of glaucoma.

Materials and methods

Eight freshly enucleated ram eyes were used for each receptor characterisation experiment. A total of 32 eyes were used in this representative series of experiments. The ciliary processes were dissected off the ciliary body and lens under a dissecting microscope and subjected to a ligand binding technique which utilised ¹²⁵iodohydroxybenzyl pindolol (¹²⁵I-HYP) as we have recently described.¹⁴ Further modifications of our technique include the use of a discontinuous sucrose gradient of 5%: 50% with added 0.1% calf serum. The binding affinities of timolol (+) propranolol, (−) propranolol, (±) propranolol, salbutamol, and practolol were investigated, and the receptors characterised by a computer-assisted nonlinear, derivative-free regression procedure, separating out mixed receptor populations (β_1/β_2) as appropriate.

Results

Fig. 1 shows a saturation curve which is characteristic of a receptor study. The radioactive material (¹²⁵I-HYP) saturates the beta receptor sites around 500 pM. Specific binding is determined from the equation: total binding−nonspecific binding.¹⁴ This figure also shows that the results are amenable to Scatchard analysis (insert), an essential prerequisite

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Fig. 1 Saturation binding curve and Scatchard analysis (insert).

for receptor analysis. The total number of receptors (β_{max}) in this representative example is 394.9 fmol/mg protein. The receptor affinity (K_a) is 440 pM ($p < 0.01$) and the correlation coefficient 93.7% for the 6 points.

Fig. 2 further confirms the presence of beta receptors. (-) Propranolol is shown to displace radioactive ^{125}I -HYP from the receptor sites

approximately 100 times more potently than (+) propranolol. This effect is typical of a functional receptor.

The computer drawn displacement curve in Fig. 3 compares the effects of salbutamol and practolol to displace ^{125}I -HYP from receptor sites. As indicated, salbutamol displaces ^{125}I -HYP more potently than practolol off the receptor sites.

Fig. 2 Relevant potencies of (-) and (+) propranolol in displacing ^{125}I -HYP from the receptors.

Fig. 3 Displacement curves of salbutamol and practolol.

Fig. 4 Displacement curves of timolol and (±) propranolol.

The computer drawn curve in Fig. 4 compares the ability of timolol and (±) propranolol to displace ¹²⁵I-HYP from receptor sites. Timolol displaces ¹²⁵I-HYP more potently than (±) propranolol off the receptor sites.

The receptor characteristics from the above graphs are summarised in Table 1 along with previous results from Trope and Clark,¹⁵ in which the iris/ciliary body diaphragm was studied. (±) Propranolol and timolol are nonselective for β_1 and β_2 receptor sites, whereas practolol and salbutamol are selective for β_1 and β_2

receptors respectively. With the revised dissection and density gradient procedures as described above we were unable to detect the presence of β_1 receptors in the ciliary processes. All the beta receptors were of the β_2 subtype.

Discussion

The discovery that beta blocking agents could lower intraocular pressure¹⁶ has led to their use for over 10 years in the medical management of primary open-angle glaucoma.³ How they work is still uncertain. Three possible mechanisms have been suggested. (i) Beta blockers may block beta receptors in the ciliary processes. (ii) They may have an effect on aqueous production through a vasoactive mechanism.^{8, 17, 18} (iii) They may have some other undetermined effect.⁸

The fact that this study confirms that large numbers of beta receptors exist in the ciliary processes strongly suggests that they influence aqueous production by blocking beta adrenergic receptor sites. This study complements the findings of other workers who, using different techniques, have also detected beta receptors in the ciliary processes.^{19, 20} It is interesting to note that the dissociation constant (Kd) for timolol obtained by the non-radio-ligand adenylyl cyclase method on human ciliary processes¹⁹ is similar to that in our study of sheep eyes.

The exact role of beta receptor blockade in aqueous formation is not known, but it has been suggested by Thomas and Epstein¹⁰ and others that beta receptors in the ciliary processes somehow provide 'tonic' activity to the inflow mechanism, and that by blocking these receptors there is a decrease in aqueous production.^{10, 21} It is possible that this effect is partially mediated by the ion transport mechanism of the epithelial cells of the ciliary processes, which is apparently cyclic-AMP-dependent.^{8, 22}

The existence of 2 subtypes of beta adrenergic receptors were first suggested by Land *et al.*²³ β_1 Receptors are found mainly in the heart and adipose tissue, and β_2 receptors mainly in the lung and liver.

Table 1 Summary of receptor characteristics

Drug	Dissociation constants (mol/l)			Receptor subtypes (% beta-1: beta-2)
	Beta-1	Beta-2	Non-selective	
(-) Propranolol	—	—	4.6×10^{-9}	—
(+) Propranolol	—	—	3.8×10^{-7}	—
Practolol*	3.2×10^{-7}	4.9×10^{-5}	—	10/90
Practolol	ND	2.0×10^{-5}	—	0/100
Salbutamol*	1.1×10^{-4}	3.0×10^{-6}	—	25/75
Salbutamol	ND	5.1×10^{-6}	—	0/100
Timolol	—	—	6.4×10^{-9}	—
(±) Propranolol	—	—	7.9×10^{-9}	—

ND=not detectable. *Iris/ciliary body diaphragm.

The use of competitive antagonistic drugs in both binding and displacement assays—coupled with computer curve fitting techniques—has enabled more precise detection and characterisation of beta adrenergic receptors and their subtypes where such exist. Similar techniques have recently been used to characterise beta receptors in nonocular sites and to detect beta receptors in albino animal eyes.^{24–26} Attempts to perform these radio-ligand techniques on the pigmented iris ciliary body diaphragm²⁷ have been partially obstructed by the presence of melanin in the tissue, as the melanin significantly binds ligand, thereby adversely affecting the results of the assay.¹⁴ This study uses a new method of separating melanin from ciliary processes.^{14,15} Our in-vitro results, as indicated above, not only confirm the presence of beta adrenergic receptors in the ciliary processes of sheep eyes, but indicate that most of these receptors appear to be β_2 in character. This finding supports the clinical evidence that β_2 receptors are probably the major receptors involved in intraocular pressure control. For example, timolol, a nonselective beta blocker, has been shown to have a more potent intraocular lowering pressure effect than metoprolol, a β_1 specific blocker. It has been suggested that this effect may be due to the presence of β_2 adrenergic blockers in the ciliary processes.²⁸

A potent new β_2 adrenergic blocking drug has recently been described.²⁹ This agent, called IPS 339, is said to be more specific than any other β_2 adrenergic blocking drug available at present.¹⁹ It has been reported to lower the intraocular pressure in normal rabbits.³⁰ Initial ligand binding studies with this drug on pigmented ciliary processes indicate that it binds potently to β_2 receptors in our membrane preparations.

By using only the ciliary processes (i.e., no iris or ciliary body) and by modifying our density gradient we have effectively removed most β_1 receptors from our membrane preparations (Table 1). Our previous study on the iris ciliary body diaphragm indicated a mixed receptor population of β_1 to β_2 receptors of 10–25%: 75–90%.¹⁵ It seems likely that most of the β_1 receptors removed with our modified technique were situated in muscle fibres and/or large vessels of the iris ciliary body diaphragm.

If beta blockers like timolol exert their effect through blockage of beta adrenergic receptors, it is acceptable to speculate that there will be an accumulation of transmitter agent in the synaptic cleft. It is conceivable that this excess noradrenaline may diffuse into the stroma of the ciliary process and by means of alpha receptors constrict the blood vessels supplying the ciliary processes. Most blood vessels are known to contain alpha receptors which cause vasoconstriction.¹³ This vasoconstriction could

then lead to a decrease in the intraocular pressure as the blood supply to the ciliary processes decreases. As a result, beta blockers may have both a beta blocker and a vascular effect which together lower intraocular pressure. Further studies on both alpha and beta receptors, particularly in human eyes, may prove of value in determining the mechanism of action of drugs currently available to treat open-angle glaucoma.

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