Binding potencies of 3 new β_2 specific blockers to β receptors in the ciliary processes and the possible relevance of these drugs to intraocular pressure control

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SUMMARY The binding potencies of 3 new β_2 blocking drugs to β receptors in the ciliary processes were studied by means of radioligand techniques. The drugs studied were IPS339, ICI118,551, and Sandoz L1 32-468. The order of potency of these drugs was IPS339>Sandoz L1 32-468>ICI118,551. The β_2 dissociation constants (KDs) for these drugs were 0.90 nM, 6.60 nM, and 55 nM respectively. These results are compared with those for other adrenergic agents, including timolol. The potential role of topical β_2 blockers in glaucoma is discussed.

Recent studies with radioligand binding and cyclic AMP techniques have revealed the presence of β_2 adrenergic receptors in pigmented ciliary processes derived from animal eyes¹ and human eyes.²

The pharmaceutical industry has developed potent β_2 specific adrenergic blockers, some of which are not yet available for clinical use. This study investigates the ability of 3 of these new β_2 blockers to bind to β_2 receptors in the ciliary processes. The agents studied are IPS339,³ ICI118,551,⁴ and L1 32-468 (Weidmann H, Engel G, (Sandoz Laboratories, Basle, personal communication). The binding potencies of these drugs are compared with those of other β adrenergic blockers which are known to lower intraocular pressure.

Materials and methods

The techniques used to separate melanin from the ciliary processes and detect β adrenergic receptors from sheep eyes with the radioligand ¹²⁵Iodohydroxybenzylpindolol (¹²⁵I-HYP) have recently been described by us.^{1,5} Recent modifications of our technique include the use of a new radioligand ¹²⁵Iodocyanopindolol (¹²⁵I-CYP), which is claimed to be even more specific for β receptors than ¹²⁵I-HYP.⁶ Owing to confusion over some of the terms used in radioligand binding studies the term dissociation

constant (Kd) is defined as representing the concentration of radioligand that binds to half the number of receptors. This value is determined from Scatchard analysis of the binding curve with either ¹²⁵I-HYP or ¹²⁵I-CYP, and is reciprocal of the slope of the line ($\frac{1}{Kd}$). This dissociation constant is a measure of the affinity of the receptors for the radioligand.⁷ It has been referred to as K_a ⁵ and K_m by some authors.⁸ The binding potencies (K_D) of the various drugs are determined by computer modelling based on the equations of Wenke,⁹ by means of a microprocessor-based nonlinear least-squares program developed by one of us for the Apple II Microprocessor (Clark B, *et al.*, paper in preparation). The drugs IPS339 and Sandoz L1 32-468 were studied with ¹²⁵I-CYP. ICI118,551 and all other drugs were studied with ¹²⁵I-HYP.¹

Results

Fig. 1. This computer drawn displacement curve indicates the potency of the β_2 specific drug ICI118,551 to displace radioligand from the β receptors. Each point represents the mean of duplicate preparations. K_D for ICI118,551 is 5.5×10^{-8} , indicating that it binds fairly potently to the β receptors in this tissue.

Fig. 2. This representative displacement curve indicates the potency of IPS339. It is interesting to note that this curve represents a 2-site fit of the raw data. This indicates that receptors other than β_2 receptors were present in the membrane prepara-

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Fig. 1 Potency of ICI118,551.

Fig. 2 2-Site fit for IPS339.

Fig. 3 Displacement curve of Sandoz L1 32-468.

tions. The K_D for the β_2 receptors is 9.0×10^{-10} . The other K_D is 9.1×10^{-9} . Further computer analysis of the data reveals a ratio of β_2 to the other receptors of 63% and 37%.

Fig. 3. This representative displacement curve indicates the potency of the β_2 specific drug Sandoz 32-468. This is a 1-site fit. K_D for Sandoz 32-468 is 6.6×10^{-9} , indicating that it binds potently to the β_2 receptors in this tissue.

Table 1. This table compares the potencies of the

above 3 β_2 specific drugs against other β blockers.¹ IPS339, Sandoz L1 32-468, timolol, and both (-) and (+) propranolol all have similar potencies, while ICI118,551 appears to be less potent than these drugs. Practolol and (-) metoprolol (both β_1 specific blockers) are noted to be the weakest drugs in this table.

A binding curve performed with ^{125}I -CYP revealed a β_{max} (maximum number of receptors) of 442 fmol/mg protein (394) with a K_D of 0.275 nM (0.44 nM). The figures for ^{125}I -HYP are shown in parentheses.¹ The theoretical and practical advantages of using ^{125}I -CYP for β -receptor estimations has recently been described by Engel *et al.*⁶

Discussion

This study shows that the 3 new β_2 adrenergic blockers IPS339, Sandoz L1 32-468, and ICI118,551 all bind potently to β_2 receptors derived from the ciliary processes of animal eyes. Sandoz L1 32-468 ($K_D=6.6$ nM) and IPS339 ($K_D=0.90$ nM) appear to be more potent than ICI118,551 ($K_D=55$ nM). It is interesting to note that 2 of these drugs, namely, IPS339 and Sandoz L1 32-468, appear to bind as potently as timolol ($K_D=6.4$ nM) to β receptor sites.¹ This is of interest because timolol is known to lower intraocular pressure (IOP) in humans and is used to treat primary open-angle glaucoma (POAG).¹⁰ IPS339 has recently been shown to lower intraocular pressure in normal rabbit eyes at least as potently as timolol.¹¹ It is therefore possible that topically applied Sandoz L1 32-468 and possibly ICI118,551 may also lower intraocular pressure.

It is interesting to note that IPS339 binds to 2 sets of receptor subpopulations (Fig. 2). Most of this binding is to β_2 receptors (63%). The radioligand ^{125}I -CYP is said not to bind to α receptors,⁶ so it seems likely that this drug binds to residual β_1 receptors present in the membranes. As β_1 receptors have not been detected

Table 1 Comparison of the potencies of 10 β blockers

Potency order	Drug name	K_D	Drug specificity
1	IPS 339*	9.0×10^{-10}	β_2 specific
2	(-) Propranolol	4.6×10^{-9}	Nonspecific
3	Timolol	6.4×10^{-9}	Nonspecific
4	Sandoz L1 32-468*	6.6×10^{-9}	β_2 specific
5	(±) Propranolol	7.9×10^{-9}	Nonspecific
6	Nadolol	2.2×10^{-8}	Nonspecific
7	ICI 118551*	5.5×10^{-8}	β_2 specific
8	(+) Propranolol	3.80×10^{-7}	Nonspecific
9	Practolol	2.0×10^{-5}	β_1 specific
10	(-) Metoprolol	2.1×10^{-5}	β_1 specific

K_D =Dissociation constant.

*This study.

with any other β blockers used by us so far, the possibility exists that IPS339 has unmasked radioligand binding to some other receptor subgroup. This requires further investigation.

There are no published reports on the effects of topical β_2 blockers on intraocular pressure in patients with POAG. A trial of such agents appears to be indicated for the following reasons. Firstly, as indicated above, β_2 blockers bind potently in vitro to β_2 receptors in animal ciliary processes. Secondly, nonspecific β blockers in eye drop form are known to produce serious cardiac side effects in elderly patients with heart disease, and to reduce resting heart rate and exercise induced tachycardia in normal patients.^{12,13} In view of the relative cardioprotective effects of β_2 blockers,¹⁴ the development of such drugs for use in elderly patients with POAG could prove to have advantages over nonspecific β blockers. The cardioprotective effect of these drugs may also have implications regarding ocular perfusion in POAG. Despite evidence to the contrary¹⁵ some workers believe that β blockers may have an effect on intraocular pressure via a vascular mechanism.¹⁶ It has been suggested that β blockers decrease aqueous production by possibly blocking β_2 receptors in uveal vasculature, producing vasoconstriction.¹⁷ If this is so, specific β_2 blockers should produce the same effect. Further studies to determine whether β receptors exist in uveal vasculature seems to be indicated.

It is interesting to note that betaxolol, a β_1 blocker, has been reported to lower the IOP.¹⁸ This drug is a potent β blocker which decreases cardiac output significantly after systemic administration.¹⁹ How it lowers the IOP in patients with POAG is not known. It is known, however, that all β_1 blockers can have β_2 blocker effects, particularly at higher doses.²⁰ The topical application of betaxolol could therefore block both β_2 receptors and any β_1 receptors that may exist in human ciliary processes. Radioligand binding studies of this drug, preferably on primate or human eyes, are indicated.

It has recently been suggested that the pigmented layer of the ciliary processes may act as a depot for topically applied β blockers. This may lead to their sustained release to ciliary process β receptors.²¹ This is an interesting theory, as we have noted that melanin derived from the ciliary processes binds radioactive ligand potently.⁵ Part of this binding is probably due to the iodine fraction of the ligand, but part is possibly also due to the pindolol fraction of the ligand. This binding by pigment may explain why a twice daily

application of topical timolol is adequate to treat glaucoma.²¹

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