Binding potencies of 3 new β₂ 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 β₂ blocking drugs to β receptors in the ciliary processes were studied by means of radioligand techniques. The drugs studied were IPS339, IC1118,551, and Sandoz L1 32-468. The order of potency of these drugs was IPS339>Sandoz L1 32-468> IC1118,551. The β₂ 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 β₂ blockers in glaucoma is discussed.

Recent studies with radioligand binding and cyclic AMP techniques have revealed the presence of β₂ adrenergic receptors in pigmented ciliary processes derived from animal eyes¹ and human eyes.² The pharmaceutical industry has developed potent β₂ specific adrenergic blockers, some of which are not yet available for clinical use. This study investigates the ability of 3 of these new β₂ blockers to bind to β₂ receptors in the ciliary processes. The agents studied are IPS339, IC1118,551,¹ 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 ¹²⁵I-Todohydroxybenzylpindolol (¹²⁵I-HYP) have recently been described by us.¹³ Recent modifications of our technique include the use of a new radioligand ¹²⁵I-Todocyanoindolol (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 (r). This dissociation constant is a measure of the affinity of the receptors for the radioligand.⁷ It has been referred to as Ka⁵ and Km by some authors.⁸ The binding potencies (K₉) 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. IC1118,551 and all other drugs were studied with ¹²⁵I-HYP.¹

Results

Fig. 1. This computer drawn displacement curve indicates the potency of the β₂ specific drug IC1118,551 to displace radioligand from the β receptors. Each point represents the mean of duplicate preparations. K₀ for IC1118,551 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 β₂ receptors were present in the membrane prepara-
above 3 β specific drugs against other β blockers. IPS339, Sandoz L1 32-468, timolol, and both (−) and (+) propranolol all have similar potencies, while IC118,551 appears to be less potent than these drugs. Practolol and (−) metoprolol (both β₁ specific blockers) are noted to be the weakest drugs in this table.

A binding curve performed with 125I-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 125I-HYP are shown in parentheses. The theoretical and practical advantages of using 125I-CYP for β-receptor estimations has recently been described by Engel et al.

**Discussion**

This study shows that the 3 new β₁ adrenergic blockers IPS339, Sandoz L1 32-468, and IC118,551 all bind potently to β₂ 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 IC118,551 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. 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).

IPS339 has recently been shown to lower intraocular pressure in normal rabbit eyes at least as potently as timolol. It is therefore possible that topically applied Sandoz L1 32-468 and possibly IC118,551 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 β₁ receptors (63%). The radioligand 125I-CYP is said not to bind to α receptors, so it seems likely that this drug binds to residual β₁ receptors present in the membranes. As β₁ receptors have not been detected

![Figure 1](image1.png)  
**Fig. 1** Potency of IC118,551.

![Figure 2](image2.png)  
**Fig. 2** 2-Sitefit for IPS339.

![Figure 3](image3.png)  
**Fig. 3** Displacement curve of Sandoz L1 32-468.

Table 1

<table>
<thead>
<tr>
<th>Potency order</th>
<th>Drug name</th>
<th>K_D</th>
<th>Drug specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IPS 339*</td>
<td>9.0×10^{-10}</td>
<td>β₁ specific</td>
</tr>
<tr>
<td>2</td>
<td>(−) Propranolol</td>
<td>4.6×10^{-9}</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>3</td>
<td>Timolol</td>
<td>6.4×10^{-9}</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>4</td>
<td>Sandoz L1 32-468*</td>
<td>6.6×10^{-9}</td>
<td>β₁ specific</td>
</tr>
<tr>
<td>5</td>
<td>(±) Timolol</td>
<td>7.9×10^{-9}</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>6</td>
<td>Nadolol</td>
<td>2.2×10^{-8}</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>7</td>
<td>IC 118,551*</td>
<td>5.5×10^{-8}</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>8</td>
<td>(±) Propranolol</td>
<td>3.80×10^{-7}</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>9</td>
<td>Practolol</td>
<td>2.0×10^{-5}</td>
<td>β₁ specific</td>
</tr>
<tr>
<td>10</td>
<td>(−) Metoprolol</td>
<td>2.1×10^{-4}</td>
<td>β₁ specific</td>
</tr>
</tbody>
</table>

K_D=Dissociation constant.

*This study.
with any other \( \beta \) 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 \( \beta_2 \) 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, \( \beta_2 \) blockers bind potently in vitro to \( \beta \) receptors in animal ciliary processes. Secondly, nonspecific \( \beta \) 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.\(^3\) In view of the relative cardioprotective effects of \( \beta \) blockers,\(^4\) the development of such drugs for use in elderly patients with POAG could prove to have advantages over nonspecific \( \beta \) blockers. The cardioprotective effect of these drugs may also have implications regarding ocular perfusion in POAG. Despite evidence to the contrary,\(^5\) some workers believe that \( \beta \) blockers may have an effect on intraocular pressure via a vascular mechanism.\(^6\) It has been suggested that \( \beta \) blockers decrease aqueous production by possibly blocking \( \beta_1 \) receptors in uveal vasculature, producing vasoconstriction.\(^7\) If this is so, specific \( \beta \) blockers should produce the same effect. Further studies to determine whether \( \beta \) receptors exist in uveal vasculature seems to be indicated.

It is interesting to note that betaxolol, a \( \beta_1 \) blocker, has been reported to lower the IOP.\(^8\) This drug is a potent \( \beta \) blocker which decreases cardiac output significantly after systemic administration.\(^9\) How it lowers the IOP in patients with POAG is not known. It is known, however, that all \( \beta \) blockers can have \( \beta_2 \) blocker effects, particularly at higher doses.\(^10\) The topical application of betaxolol could therefore block both \( \beta_1 \) receptors and any \( \beta_2 \) 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 \( \beta \) blockers. This may lead to their sustained release to ciliary process \( \beta \) receptors.\(^11\) This is an interesting theory, as we have noted that melanin derived from the ciliary processes binds radioactive ligand potently.\(^3\) 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.\(^12\)

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References