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ICI 118,551: an effective ocular hypotensive agent with selectivity for the ciliary process β_2-adrenoceptor and with minimal cardiac side effects

James A. Nathanson

Department of Neurology, Harvard Medical School, and Neuropharmacology Research Laboratory, Massachusetts General Hospital, Boston, Massachusetts 02114, U.S.A.

1 Prior biochemical studies have shown that the ciliary process epithelium, which is involved in the secretion of aqueous humour, is rich in β-adrenoceptors with pharmacological characteristics similar to those of the β_2 subclass.

2 The present experiments demonstrate that the β-adrenoceptor antagonist, ICI 118,551, is a potent inhibitor of isoprenaline-stimulated adenylate cyclase activity measured in broken cell preparations of rabbit ciliary process.

3 In rabbit cardiac muscle, however, ICI 118,551 is a relatively weak antagonist of isoprenaline-stimulated adenylate cyclase, being approximately 100 fold less potent than the non-selective β-adrenoceptor antagonist, timolol.

4 ICI 118,551 is also less potent than timolol in inhibiting isoprenaline-sensitive adenylate cyclase of rabbit lung.

5 ICI 118,551 applied topically to eyes of unanaesthetized rabbits causes a dose-dependent decrease in intraocular pressure. Furthermore, in a blind crossover study in rabbits, topically applied ICI 118,551 decreased intraocular pressure for more than 6 h and was more effective than an identical dose of the clinically effective anti-glaucoma agent, timolol.

6 Systemic absorption from topically-applied timolol, but not ICI 118,551, is sufficient to alter cardiac response to subcutaneous administration of isoprenaline. Furthermore, dose-response studies, using direct systemic administration of the two β-adrenoceptor antagonists, revealed that ICI 118,551 is about 60 times less potent than timolol in blocking isoprenaline-induced cardio-acceleration.

7 ICI 118,551, applied to one eye, causes a decrease in intraocular pressure in the contralateral eye, and systemic administration of ICI 118,551 results in decreased intraocular pressure in both eyes, data indicating that at least part of the ocular hypotensive effect of topical ICI 118,551 is mediated through systemic absorption.

8 These findings provide biochemical and physiological evidence that selective β_2-adrenoceptor blockers such as ICI 118,551, used topically or systemically, may be useful as ocular hypotensive agents with decreased cardiac side-effects.

Introduction

Recent biochemical studies of isoprenaline-sensitive adenylate cyclase (Nathanson, 1980; 1981b; Cepelik & Cernohorsky, 1981) and β-adrenoceptor binding (Trope & Clark, 1982) indicate that the majority of β-adrenoceptors in the ocular ciliary process have pharmacological characteristics similar to (or possibly identical with) those of the β_2-adrenoceptor subtype. Physiological experiments (Nathanson, 1981a; Colasanti & Trotter, 1981; Green & Mayberry, 1983) also support this notion. Tissue fractionation studies of the ciliary process indicate that the ciliary β-adrenoceptors are particularly abundant in epithelial cell fractions which contain the secretory cells involved in aqueous humour formation (Nathanson, 1980). In light of physiological experiments which suggest that certain of the actions of non-selective β-adrenoceptor antagonists (e.g., timolol) in decreasing intraocular pressure (IOP) may be through

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decreased aqueous humour formation, it has been suggested that blockade of ciliary process β-adrenoceptors may, at least in part, play a role in the observed clinical effects of timolol in decreasing IOP (for review, see Neufeld *et al.*, 1984).

The characterization of ciliary β-adrenoceptors as similar to β_2 suggests that potent and selective β_2-antagonists should be effective in decreasing IOP and, at the same time, have fewer side effects on tissues containing β_1-receptors, such as heart, than non-selective β-adrenoceptor antagonists. Previous biochemical studies have shown that some older β_2-antagonists, such as butoxamine and H35/25, which were previously considered to be fairly selective, show only a low degree of selectivity for β_2-versus β_1-receptors (Minneman *et al.*, 1979a).

A newer antagonist, IPS 339, has been reported to demonstrate much greater β_2 selectivity *in vitro* (Minneman *et al.*, 1979b; Nathanson, 1980) and, *in vivo*, this compound has been found to have ocular hypotensive effects in rabbits similar to those of timolol (Nathanson, 1981a). However, there has been some uncertainty, from physiological studies in non-ocular tissues, about whether the degree of β_2-adrenoceptor selectivity shown by IPS 339 *in vitro*, is always manifest *in vivo* (Imbs *et al.*, 1977; Holmberg *et al.*, 1980; O'Donnell & Walduck, 1981).

Recently, Bilski *et al.*, (1980) have described a new, potent β-adrenoceptor antagonist, ICI 118,551 (erythro-DL-1- (7-methylindan-4-yloxy) -3-isopropylaminobutan-2-ol), which appears to demonstrate high β_2 selectivity in studies of isolated tissue preparations of guinea-pig trachea and atria (O'Donnell & Wanstall, 1980). The present experiments were designed for three purposes: first, to determine, *in vitro*, using measurements of isoprenaline-stimulated adenylate cyclase activity, whether ICI 118,551 demonstrates potency and selectivity for the ciliary process β-adrenoceptor as compared to the cardiac β-receptor; second, to determine, *in vivo*, whether topically-applied ICI 118,551 can decrease IOP, and to what degree it affects cardiac function when given systemically to unanaesthesized rabbits; and third, to determine whether ocular administration of β-adrenoceptor antagonists, such as ICI 118,551, can (through incidental systemic absorption) affect peripheral cardiovascular function and whether systemic administration of such agents can alter ocular physiology.

Methods

Measurement of isoprenaline-sensitive adenylate cyclase

Eyes and ventricular heart tissue were obtained from male, 2–4 kg, New Zealand white rabbits which were anaesthesized and killed with an inhalation overdose of diethyl ether. In other experiments, we have found that ether anaesthesia, itself, does not affect the pharmacological characteristics of the isoprenaline-sensitive adenylate cyclase in the ciliary process. To obtain ciliary process, following enucleation, each eye was opened through a circumferential incision 3 mm posterior to the limbus, and the vitreous was bluntly dissected from the posterior surface of the lens. The opened, anterior third of the eye was placed, cornea-down, in a solution of artificial aqueous humour (in $mmol\,l^{-1}$:NaCl 130, KCl 2.7, NaH_2CO_3 18.3, $MgCl_2$ 1.33, $CaCl_2$ 1.5, glucose 10 and HEPES 10, pH 7.4), and the lens was removed by dividing the zonules at the level of the lens capsule. The villus-like ciliary processes were transected at their attachments to the ciliary body, removed, washed in NaCl 150 mM, and homogenized ($10\,mg\,ml^{-1}$) by hand in an all-glass homogenizer in Tris maleate buffer 6 mM, pH 7.4. Cardiac muscle was obtained from the tip of the left ventricle, minced, and homogenized as above at a concentration of 25 or 50 mg wet weight per ml. In some experiments, isoprenaline-sensitive adenylate cyclase activity was also determined in rabbit lung, using, as a source of enzyme, an homogenate ($25-50\,mg\,ml^{-1}$) of a peripheral wedge of pulmonary tissue.

Adenylate cyclase activity was measured in test tubes containing (in 0.3 ml) Tris maleate 80 mM, pH 7.4, theophylline 10 mM, EGTA 0.5 mM, $MgCl_2$ 8 mM, GTP 0.03 mM, ATP 2 mM and tissue homogenate; plus or minus test substances as indicated. (In some experiments, a washed P_2 fraction sedimenting at $10^5\,g$ was used, with similar results.) The amount of GTP present was that which (as determined in preliminary experiments) gave optimal enzyme activity. The enzyme reaction (4 min at 30 °C) was initiated by addition of ATP, stopped by heating to 90 °C for 2 min, and then centrifuged at $1000\,g$ for 15 min to remove insoluble material. Adenosine 3′:5′ cyclic-monophosphase (cyclic AMP) in the supernatant was measured by protein binding assay (Brown *et al.*, 1972). Under the assay conditions used, adenylate cyclase activity was linear with respect to time and protein concentration, and cyclic nucleotide phosphodiesterase activity was almost completely inhibited. Other experiments indicated that heat inactivation (versus other methods of reaction termination) did not affect the results obtained. Protein concentration was determined by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Activation constants (K_a) and IC_{50} values were determined, where necessary, with a computerized sigmoidal curve fitting programme (Rodbard & Hutt,

1974) utilizing 8–12 data points per dose-response curve. Inhibitory constants (K_i) for timolol and ICI 118,551 were calculated from the equation (Chen & Prusoff, 1973), $K_i = (IC_{50}) / (1 + s/K_a)$; where IC_{50} was the concentration of antagonist necessary to give 50% inhibition of activity in the presence of isoprenaline, S was the concentration of isoprenaline present, and K_a was the concentration of isoprenaline (3.8×10^{-7} M) necessary for half-maximal activation of ciliary process adenylate cyclase activity.

All common reagents were from Sigma Chemical Company. ICI 118,551 was obtained from ICI and timolol from Merck and Co.

Effects on intraocular pressure

Methods were similar to those described previously (Nathanson, 1981a). Briefly, male, 2–4 kg, New Zealand white rabbits were housed under standard conditions and exposed to a 12 h light-dark cycle. Intraocular pressure (IOP) was measured with a Perkins applanation tonometer after topical anaesthesia with 0.4% benoxinate (and 0.25% sodium fluorescein). The tonometer had previously been calibrated by measuring pressure in enucleated rabbit eyes which were connected to a manometer system that allowed known, graded variations in pressure to be applied. A large number of preliminary IOP readings were made in order to accommodate the animals to the measurement procedure. These readings indicated reproducible control pressures comparable to those found by others in rabbits (e.g., Bhattacherjee, 1971). To minimize possible diurnal pressure variations, measurements were made at the same time of day for both drug and control eyes. For dose-response studies with ICI 118,551, a group of 8 rabbits received, in the conjunctival sac of the left eye, in a volume of 50 µl, a given dose, mixed in phosphate buffered saline (PBS), pH 7.4. Similar results were also obtained when the drugs were given in two 25 µl doses, 5 min apart. IOP prior to drug administration was then compared with the pressure, measured in both the left and right eyes, 1 h after drug administration; other studies (see Figure 3) indicated that pressure decrease was maximal at 1 h after drug administration. After a period of three days, rabbits were then given the next higher dose. Preliminary experiments indicated that pressure returned to normal within 24 h and that no significant desensitization to the effects of ICI 118,551 would occur if the drug were administered at intervals of three days for the duration of the dose-response studies.

For time-course studies comparing the IOP effects of ICI 118,551 with timolol (Figure 3), experiments were carried out in a blind fashion by two experimenters, one applying the drugs (or placebo) and the second measuring IOP. The second experimenter had no knowledge of which drugs (or placebo) were applied until the end of the entire study. In these studies, six rabbits (not previously treated with any drugs) received either 1% ICI 118,551 (in PBS) or 1% timolol (in PBS) in the left or right eye. The contralateral eye received PBS alone. IOP measurements were made on both eyes at 0, 1, 2, 3, 4, and 6 h after application. By 10 h after application, IOP in almost all eyes had returned to normal. Two days later, the same six rabbits again received ICI 118,551 or timolol, this time to the eyes previously treated with PBS, and IOP was measured as above. This procedure was repeated again on subsequent alternate days, such that by the end of the experiment each of the 12 eyes had received one dose of each of the two drugs, and no eye had received the same drug more than once. Using this alternate day, alternate eye procedure, there was a minimum of 4 days between drug applications to a given eye. As an additional control, during one of the alternate days, each rabbit received placebo in both eyes. Measurements of IOP on these control days were averaged for all six animals (12 eyes) and served as the control values shown in Figure 3. In addition to those rabbits above, two others received no drugs but only PBS for the entire duration of the experiment. IOP measurements for these rabbits, recorded on the same schedule as for those described above, revealed no significant change in baseline IOP during the course of the blind study.

For those studies investigating effects of systemic administration of ICI 118,551 on IOP, each of a group of 5 rabbits was given a single subcutaneous 30 µg kg^{-1} dose (mixed in PBS at a concentration of 100 µg ml^{-1}). Pressure in both eyes was then measured at intervals of 30 min, 1, 2, 3, 4, and 6 h and compared with pre-drug control. Other experiments, described below, indicated that the administered dose of 30 µg caused minimal chronotropic effects on the heart.

Effects on cardiac function

Studies of cardiac function were carried out with unanaesthesized, restrained rabbits of similiar age, sex and species as those described above. Monopolar needle electrodes (22 g) were placed subcutaneously, and electrocardiographic activity was monitored and recorded continuously with a standard clinical ECG unit. Rabbit heart rate (determined by the QRS spike complex) was measured continually, and averaged and recorded automatically every 10 s, using an integrating computer linked to a chart recorder.

Various doses of timolol or ICI 118,551, dissolved

in PBS, were administered through previously placed subcutaneous catheters (21g). The effects of these agents were measured on (a) baseline ECG activity and heart rate, and (b) on their ability to inhibit (30 min after their injection) the cardio-acceleratory effect of 3 $\mu g\ kg^{-1}$ of isoprenaline injected through a separate subcutaneous catheter. It had been determined previously, in isoprenaline dose-response experiments in several rabbits, that, for the subcutaneous route of injection, this dose of isoprenaline approximated the EC_{50} amount for cardio-acceleratory activity in the rabbit. Cardio-acceleration was quantitated by integrating the increase in heart rate (over starting rate) for a 30 min period beginning at the time of isoprenaline injection. The degree of inhibition resulting from a particular dose of antagonist was then determined by comparing the integrated cardio-acceleration due to isoprenaline following a dose of antagonist compared with the integrated cardio-acceleration of isoprenaline following saline injection at the beginning of a particular experiment. In these studies on cardiac function, only one antagonist

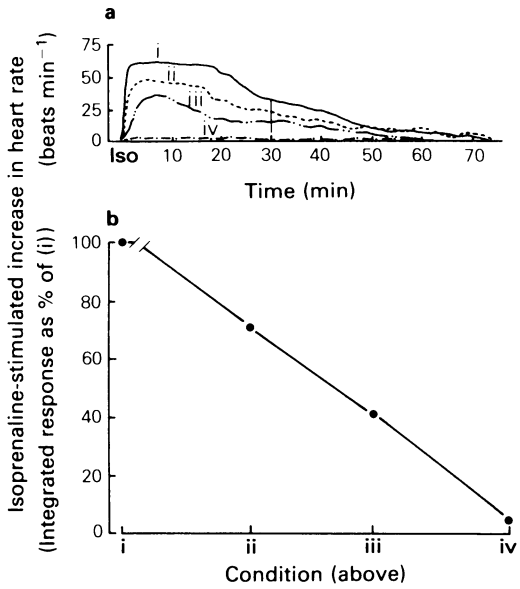

Figure 1 Effect of various subcutaneous doses of timolol on blocking cardio-acceleration due to subcutaneous injection of isoprenaline (3 $\mu g\ kg^{-1}$). In (a) four superimposed tracings show the increase (in beats min^{-1}) above basal heart rate (average 180 beats min^{-1}) due to injection of isoprenaline at time 0. In tracing (i), isoprenaline alone was injected. In tracing (ii), 1 $\mu g\ kg^{-1}$ of timolol had been injected 30 min before isoprenaline. In (iii), 3 $\mu g\ kg^{-1}$ of timolol had been pre-injected; and, in (iv), 10 $\mu g\ kg^{-1}$ of timolol had been pre-injected. In (b), the areas under curves (ii) (iii), and (iv) have been plotted as a percentage of the area under curve (i).

was used per rabbit per experiment, and the same rabbit was not used again for at least one week. Within a given experiment, the interval between repeated doses of isoprenaline was 90 min.

The above procedure is illustrated in Figure 1, showing the effect of increasing doses of timolol on the cardio-acceleratory effect of isoprenaline. In Figure 1a, curve (i) shows the effect of 3 $\mu g\ kg^{-1}$ isoprenaline alone in elevating heart rate above baseline. In curve (ii), in which a 1 $\mu g\ kg^{-1}$ dose of timolol had been given 30 min before the administration of isoprenaline, the cardio-acceleratory response was blunted. Higher doses of timolol, (iii) and (iv) progressively blocked the isoprenaline response. Other experiments indicated that this blockade was not due to desensitization to repeated doses of isoprenaline. In Figure 1b the areas under curves (ii)-(iv) shown in Figure 1a have been plotted as a percentage of the area seen with isoprenaline alone (area under (i)). Data of the type shown in Figure 1b were used as the basis for the comparisons described in the Results section.

For those studies investigating the effects of ocular administration of ICI 118,551 or timolol on systemic cardiac response, rabbits were set up for cardiac monitoring as described above. First, a 3 $\mu g\ kg^{-1}$ dose of isoprenaline was given subcutaneously and the resultant cardio-acceleration recorded. Then, approximately 2.25 h later, rabbits were given either 1% timolol or 1% ICI 118,551 by topical ocular administration (in two 25 μl doses, 5 min apart). Finally, 1 h later, rabbits were rechallenged with isoprenaline, given subcutaneously and the cardiac response measured.

Results

Biochemical studies: selectivity of β-adrenoceptor antagonists for ciliary process versus heart

In each tissue (ciliary process or cardiac muscle), ICI 118,551 and timolol were evaluated separately for their ability to block the activation of isoprenaline-stimulated adenylate cyclase activity. For each blocker, dose-response curves were constructed by varying the concentration of antagonist against a fixed concentration (10 μM isoprenaline) of agonist. The IC_{50} values determined from these curves, together with the K_a for isoprenaline in each tissue, were used to derive the inhibitory constants (K_i) for each compound. The values shown in Table 1 are the mean K_i s ($\pm$ s.e.mean) determined from 4 to 6 separate experiments. In the absence of isoprenaline, neither ICI 118,551 nor timolol stimulated enzyme activity; thus, these antagonists did not have any activity as partial agonists.

Table 1 Calculated K_i of ICI 118,551 and timolol for inhibition of rabbit ciliary process and heart isoprenaline-stimulated adenylate cyclase

Antagonist	K_i (μM) *for adenylate cyclase*		*Ratio* K_i *Heart: Ciliary process*
	Ciliary process	*Heart*	
ICI 118,551	0.0043 ± .0013	2.1 ± .52	480.0
Timolol	0.0042 ± .0011	0.019 ± .013	4.5

Values for K_i were calculated from IC_{50} values which were derived from dose-response curves for antagonists (see text). Results shown are the means (± s.e.mean) from 4 to 6 separate experiments. Also shown are the ratio of K_i s calculated from the values (Heart: Ciliary process) in the first two columns.

In the ciliary process, timolol ($K_i = 4.2 \times 10^{-9}$ M) was nearly equipotent to ICI 118,551 ($K_i = 4.3 \times 10^{-9}$ M) in inhibiting isoprenaline stimulation of enzyme activity. In cardiac muscle, however, ICI 118,551 ($K_i = 2.1 \times 10^{-6}$ M) was approximately 100 times less potent than timolol ($K_i = 1.9 \times 10^{-8}$ M) in blocking isoprenaline-sensitive activity. Thus, ICI 118,551 was much more selective than timolol for inhibiting the ciliary process β-adrenoceptor. This selectivity is quantified in the last column of Table 1, which shows, for each compound, the ratio of K_i s between the two tissues. ICI 118,551 was, on the average, more than 400 times as effective in blocking isoprenaline-stimulated activity in the ciliary process as in the heart. Timolol, on the other hand, was only about 4 to 5 times more potent in the ciliary process than in the heart. In preliminary experiments using human tissue from postmortem donor eyes, we have found that the selectivity of ICI 118,551 for the ciliary process versus heart β-adrenoceptor is also seen in human tissues.

Biochemical studies: inhibition of rabbit lung isoprenaline-sensitive adenylate cyclase

ICI 118,551 also showed selectivity for rabbit ciliary process versus lung isoprenaline-stimulated adenylate cyclase. In the lung, the K_i of ICI 118,551 for inhibition of isoprenaline-stimulated enzyme activity was $5.1 \pm 1.9 \times 10^{-7}$ M and the ratio of potency for ciliary process versus lung was about 150. This compares with timolol, which, in the lung, had a K_i of $2.6 \pm 1.9 \times 10^{-8}$ M and a potency ratio relative to ciliary process of about 6. This apparent selectivity of ICI 118,551 for ciliary process versus lung could be due (a) to a difference in the characteristics of β_2-adrenoceptors in these two tissues or (b), more likely,

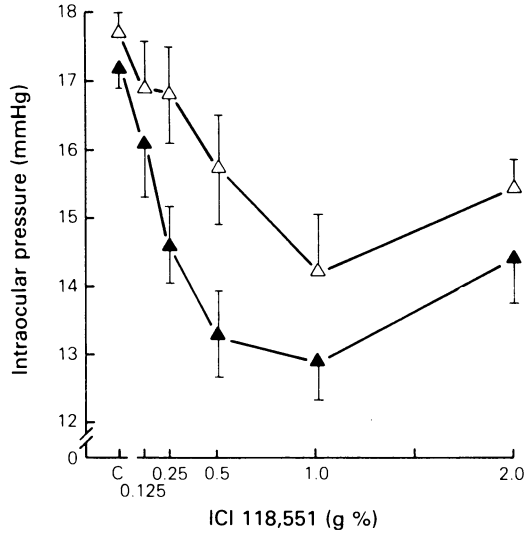

Figure 2 Effect of topical ocular administration of ICI 118,551 (at concentrations shown) on intraocular pressure in rabbits (n =8). Drug (50 μl) was administered in left eye (▲) and pressure measured in both eyes 1 h after administration; right eye (△). See text for details of procedure. Vertical bars here and in the remaining figures show s.e.mean.

to the fact that, in certain species, lung tissue may contain a mixture of β_2- and β_1-adrenoceptors (Minneman *et al.*, 1979b).

Ocular studies: dose-dependent effects of topical ICI 118,551 on intraocular pressure

Topical ocular administration of ICI 118,551 in rabbits resulted in a dose-dependent decrease in IOP. Figure 2 shows the mean values, measured 1 h after administration, for a group of 8 animals. (As will be shown in Figure 3, below, the greatest decrease in IOP occurred 1 h post-administration; therefore, this particular time interval was chosen for the dose-response studies.) Compared with pre-drug control, ICI 118,551 caused a maximal 4.2 mm Hg decrease in IOP at a concentration of 1%. Decreases in IOP were significant at ICI 118,551 doses of 0.25% ($P<0.02$), 0.5% ($P<0.002$), 1.0% ($P<0.002$), and 2.0% ($P<0.002$).

It was of interest that topical administration of ICI 118,551 to one eye also caused a decrease in IOP in the contralateral eye. As shown in Figure 2, the dose-dependency of this contralateral effect was similar to that seen in the ipsilateral eye and the magnitude of this effect was about 60% of that seen in the ipsilateral eye. For the contralateral eye, statistically significant IOP decreases (relative to pre-drug

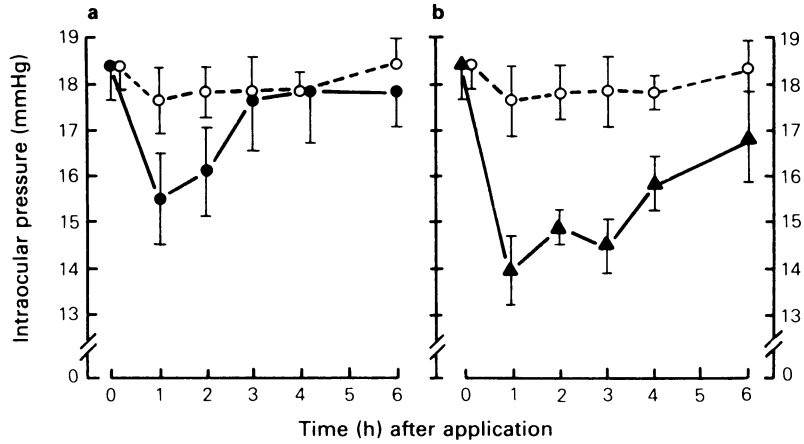

Figure 3 Time-course effects of topically applied 1% timolol (●) (a), or 1% ICI 118,551 (▲) (b), compared with vehicle (○), on ipsilateral intraocular pressure in rabbit eyes. Groups ($n = 12$) are as described in text.

contralateral resting IOP) occurred at doses of 1% ($P < 0.05$) and 2% ($P < 0.002$). Additional experiments, to be described below, suggest that this contralateral effect of ICI 118,551 is due to systemic absorption.

During the course of the above dose-response study, there were no observed effects on pupillary diameter nor on conjunctival inflammation.

Ocular studies: time course effects of topical ICI 118,551 vs timolol on intraocular pressure

In these time-course experiments, ICI 118,551 was compared with timolol, a non-selective β-adrenoceptor antagonist that is currently the β-adrenoceptor blocker of choice for clinical use in glaucoma (Zimmerman & Boger, 1979). Figure 3 shows the mean IOP for the 6 h following topical administration of either ICI 118,551 or timolol to the ipsilateral eye. As detailed in the Methods section, for this blind cross-over study, $n = 12$. Figure 3 demonstrates that, after topical administration, timolol caused a decrease in IOP similar to that which Nathanson (1981) and others (for review, see Neufeld *et al.*, 1984) have observed, previously, in rabbits. In the present experiments, maximal decrease occurred at 1 h and 2 h following administration, with a return to pre-drug readings by 4 h.

Compared with timolol, ICI 118,551 produced both a greater and more prolonged decrease in IOP. Pressure decrease was maximal during the first 3 h ($P < 0.01$ versus control for hours 1, 2, and 3; unpaired t test), and a half maximal effect was still present by 4 h ($P < 0.02$). By 6 h, pressure was still decreased but not significantly so. At 3 h, the hypotensive effect of ICI 118,551 was significantly greater ($P < 0.01$) than that due to timolol.

In these time course studies (and similar to what was found in Figure 2), ICI 118,551 also caused a decrease in IOP in the contralateral eye (data not shown). This decrease had the same general time course as that in the ipsilateral eye, and was significantly less than control at 1, 2, and 3 h ($P < 0.01$). Timolol also caused a decrease in mean pressure in the contralateral eye at 1 and 2 h, but this decrease was not statistically significant. These contralateral effects have previously been reported for timolol

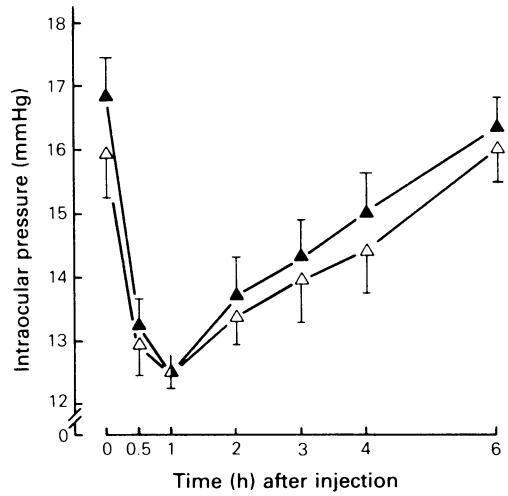

Figure 4 Time-course effects of subcutaneous injection (30 μg kg^{-1}) of ICI 118,551 on intraocular pressure ($n = 5$); right eye (▲); left eye (△).

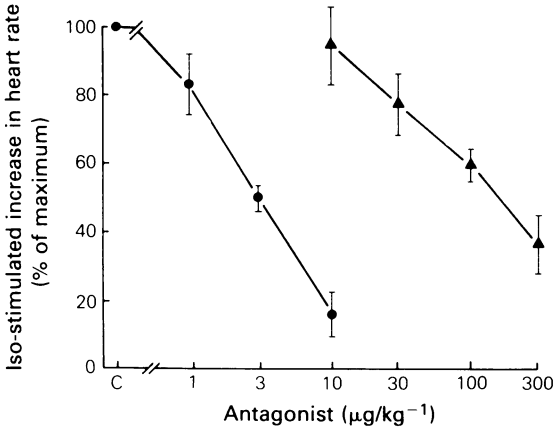

Figure 5 Effect of timolol (●) or ICI 118,551 (▲) in blocking the increase in heart rate caused by injection of 3 μg kg^{-1} of isoprenaline HCl. All drugs were give by the subcutaneous route. Rate is expressed as a percentage of the increase (over resting heart rate = 180 beats min^{-1}) caused by isoprenaline in the absence of any antagonist. See Figure 1 and text for details for procedure.

(Zimmerman & Boger, 1979) and IPS 339 (Nathanson, 1981) and, as described below, appear to be due to systemic absorption of the drug.

Ocular studies: effects of sytemic administration on intraocular pressure

Because of the contralateral decreases in IOP observed after ipsilateral topical administration, it appeared possible that drug was being absorbed systemically, either locally or through oral ingestion of tears conducted via the nasolacrimal duct. Therefore, it was of interest to determine if direct systemic administration of ICI 118,551 would result in a decrease in IOP. The 30 μg dose was chosen on the basis of cardiac studies (described below) which indicated that this dose caused very little effect on cardiac β-adrenoceptor-mediated responses.

Figure 4 shows that subcutaneous administration of ICI 118,551 caused a rapid and substantial decrease in IOP in both eyes. This decrease was near maximum at 30 min, maximum at 1 h and IOP returned to normal by 6 h. With systemic administration, the magnitude of ocular hypotension observed through the first 4 h was similar to that seen with the best topical dose of ICI 118,551.

Cardiac studies: effects of systemic administration of β-adrenoceptor antagonists

When injected subcutaneously, neither timolol nor IPS 118,551 caused any observable changes in the shape of the rabbit electrocardiogram, nor did they cause any atrial or ventricular arrhythmias during the course of the study. However, both compounds were effective in blocking isoprenaline-stimulated cardio-acceleration (Figure 5, curves constructed as described in methods and in Figure 1). The mean IC_{50} for timolol was 2.9 ± 0.2 (s.e.mean) μg kg^{-1} while the mean IC_{50} for ICI 118,551 was 176 ± 41 μg kg^{-1}. Thus, ICI 118,551 was about 60 times less potent than timolol in affecting cardio-acceleration. This decreased potency of ICI 118,551 in the heart relative to timolol is consistent with the previously described biochemical studies (Table 1) which showed that ICI 118,551 was about 100 times less potent than timolol in activating cardiac isoprenaline-sensitive adenylate cyclase.

Cardiac studies: effects of ocular administration of β-adrenoceptor antagonists on cardiac function

As described previously, data from the experiments

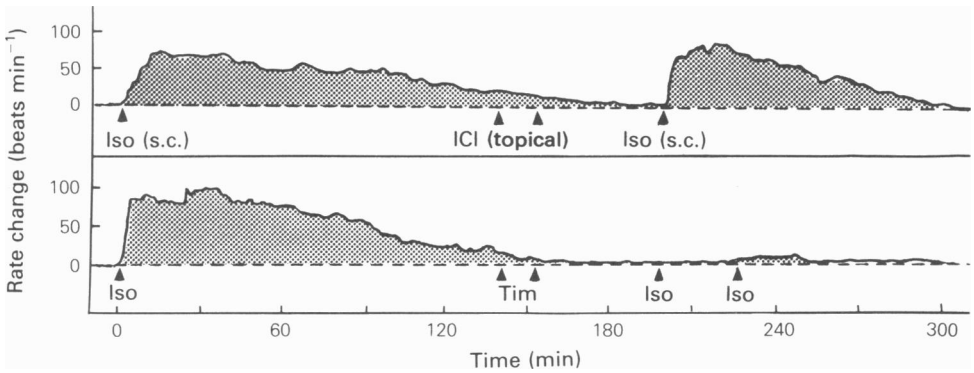

Figure 6 Effect of topical ocular administration of ICI 118,551 (a) or timolol (b) in blocking the cardio-acceleratory effects of subcutaneously administered isoprenaline. Resting heart rate (in the absence of isoprenaline) averaged 180 beats min^{-1}. ISO: isoprenaline; Tim: timolol; ICI: ICI 118,551. See text for details.

shown in Figure 2 and 3 (indicating contralateral decreases in IOP from ipsilateral application) suggest that systemic absorption of drug may occur following topical ocular administration. To investigate this possibility further, experiments were set up to measure the effects of a topical ocular dose of β-adrenoceptor antagonist on cardiac response to systemically administered isoprenaline. The ocular dose chosen (1% drug given in two 25 μl applications, 5 min apart) was that which was used in the time course IOP studies in Figure 3. As shown in Figure 6, an animal was first given a subcutaneous dose of isoprenaline (3 $\mu g\,kg^{-1}$) and the resultant cardio-acceleration measured. The animal was next given a topical ocular dose of the antagonist and 60 min later, was rechallenged with a subcutaneous dose of isoprenaline. As illustrated in Figure 6a, ocular administration of ICI 118,551 had no effect on cardiac response. However, ocular administration of an equivalent dose of timolol (separate animal, Figure 6b), caused complete blockade of cardiac response to isoprenaline. In this latter animal, response to a third injection of isoprenaline was still almost completely blocked 30 min later. Responses similar to those shown in Figure 6 were seen in all animals tested ($n=4$). These experiments indicate that substantial systemic absorption of β-adrenoceptor antagonist occurs following topical ocular administration. In fact, based on the data shown in Figure 5, it can be estimated that the topical application of 1% ($10\,mg\,ml^{-1}$) timolol results in a systemic concentration equivalent to that resulting from a subcutaneous injection of 10 $\mu g\,kg^{-1}$ or more.

Discussion

The above experiments demonstrate that, on the basis of both biochemical and physiological criteria, ICI 118,551 has much greater selectivity for the ciliary process (versus heart) β-adrenoceptor than does (−)-timolol. Indeed, ICI 118,551 shows a greater ratio of selectivity for the ciliary process versus heart than any other β_2-selective antagonist (butoxamine, H35/25, IPS 339, α-methyl-propranolol) we have yet tested (Nathanson, 1980; 1981b; and unpublished). The present data, together with results observed previously with IPS 339, suggest that it may be of interest to carry out additional investigations of the effects of potent and specific β_2-adrenoceptor antagonists in experimental models of glaucoma.

In the present study, the K_i of ICI 118,551 for isoprenaline-stimulated adenylate cyclase in the ciliary process ($4.3 \pm 1.3 \times 10^{-9}$ M) was similar to that (about 2×10^{-9} M) which O'Donnell & Wanstall (1980) obtained in studies of muscle tone in guinea-pig trachea contracted with the β_2-agonist, fenoterol. Although there have been, as yet, no other published studies of the effects of ICI 118,551 on isoprenaline-sensitive adenylate cyclase, Harden & McCarty (1982) have reported that, in binding studies using iodohydroxypindolol, ICI 118,551 had a calculated K_i of 2×10^{-9} M in 1321N1 astrocytoma cells, which contain a majority of β_2-receptors. In the present experiments, which used isoprenaline-stimulated adenylate cyclase activity in broken cell preparations, the calculated degree of selectivity of ICI 118,551 for rabbit ciliary process versus heart was somewhat greater than the degree of selectivity that has been reported in physiological studies comparing guinea-pig uterus versus atrium (Bilski *et al.*, 1979) or in guinea-pig trachea versus atrium (O'Donnell & Wanstall, 1980). Such differences in selectivity may be related to the particular tissue type and species studied or to the effects of drug access and metabolism present in the intact tissue studies. In other studies, ICI 118,551 has been reported to have a membrane stabilizing action similar to that of propranolol (Bilski *et al.*, 1980).

Data from ocular physiology studies suggest that β-adrenoceptor antagonists act to decrease IOP by affecting secretion in the ciliary process (Neufeld *et al.*, 1984). Studies with isolated ciliary process *in vitro* further suggest that this effect may be direct, either on the ciliary epithelium of ciliary vasculature (Green & Mayberry, 1983). Because significant levels of adrenoceptor-active drugs appear in the aqueous humour after topical ocular administration (e.g., Mindel *et al.*, 1984), it has been thought that topically-applied drug, after penetrating the cornea, reaches the ciliary process by diffusion through the aqueous humour. However, for this route to be effective, the rate of diffusion toward the surface of the ciliary process would have to exceed the rather considerable rate of fluid outflow away from the surface of the ciliary epithelium.

The results of the present studies raise the possibility that β-adrenoceptor antagonists, applied topically to the eye, may also reach the ciliary process through the blood. First, as described in Figures 2 and 3, application of drug to one eye results in a decrease in IOP in both the ipsilateral and contralateral eye. Second, as demonstrated in Figure 6, topical ocular administration results in pharmacologically significant systemic blood levels. Third, as shown in Figure 4, systemic administration of drug (at a concentration similar to that which may effectively result from ocular application) decreases IOP to a level similar to that resulting from topical ocular administration.

With unilateral ocular administration of either timolol or ICI 118,551, we observed a greater decrease in IOP in the ipsilateral than in the contralateral eye (Figure 2); therefore, it is possible that both of the above-described routes of access to the ciliary process could occur during topical administration.

Furthermore, the above results, by themselves, do not rule out the possible influence of extraocular sites in the mechanism of action of ICI 118,551 or timolol in decreasing IOP.

Note added in proof

Consistent with the present results, Trope & Clark (*Br. J. Ophthalmol.*, **68,** 245–247, 1984) and Schmitt *et al.* (*Graefés Arch. Clin. exp. Ophthalmol.*, **221,** 167–170, 1984) have recently shown that ICI 118,551 can bind with high affinity to ciliary process membranes.

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