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Selective β_2-adrenoceptor antagonists: derivatives of ICI 118,551 and a binary aryloxypropanolamine

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Abstract—Recent studies indicate that selective β_2-adrenoceptor antagonists may be of use in the treatment of glaucoma. ICI 118,551, its desmethyl-, didesmethyl-, and ethyl- analogues, and a putative highly β_2-selective binary aryloxypropanolamine (4d) have been evaluated for their ability to inhibit β-adrenergic-stimulated adenylate cyclase activity in rabbit ciliary process and heart. Potency ratios (K_i heart/K_i ciliary process) were 440 for ICI 118,551; 9·3 for the desmethyl analogue; 8·2 for the didesmethyl analogue; 720 for the ethyl analogue; and 11 for the binary aryloxypropanolamine. The values for the ethyl derivative of ICI 118,551 indicate that it is the most oculoselective β-adrenoceptor antagonist yet reported.

Until recently, clinical applications for selective β_2-adrenoceptor antagonists have been relatively limited and, perhaps as a consequence, the number of highly selective β_2-blockers available has been few. In the past several years, biochemical studies have shown that the aqueous humor-secreting ciliary process is highly enriched in β_2-adrenoceptors receptors (Nathanson 1980, 1981a; Cepelik & Cernohorsky 1981; Trope & Clark 1982; Elena et al 1984; Schmitt et al 1984; Wax & Molinoff 1987), and physiological studies have demonstrated that selective β_2-antagonists can alter ciliary process permeability and decrease intraocular pressure (Nathanson 1981b, 1984, 1985a; Green & Mayberry 1983; Woodward et al 1986). Because of this, β_2-blockers show promise for use in the treatment of glaucoma, manifesting fewer cardiac side effects than non-specific β-blockers such as timolol. Recent studies also suggest that β_2-blockers may have use in the regulation of brain water by the blood-brain and blood-CSF barriers (Nathanson 1982, 1983).

Previous in-vitro receptor studies have shown that traditional β_2-blockers such as butoxamine and H35/25 are only modestly oculoselective, having selectivity ratios (K_i for heart/K_i for ciliary process) of less than ten (Minneman et al 1979; Nathanson 1985b). IPS 339 and α-methyl-propranolol are significantly more selective, and the most selective agent yet described is ICI 118,551 (Bilski et al 1979), with a selectivity ratio of about 400 (Nathanson 1984, 1985b).

When ICI 118,551 was synthesized, two other derivatives (*p*-ethyl and desmethyl ICI 118,551) were also described, neither of which has yet been evaluated for possible clinical use (Tucker 1979). Because of the increasing need to find selective β_2-adrenoceptor blockers, we have now evaluated and compared the ability of these derivatives (plus a didesmethyl *t*-butyl derivative of ICI 118,551) to block β-adrenoceptors in the ocular ciliary process and cardiac ventricle. We also evaluated the oculoselectivity of a recently synthesized binary aryloxypropranolamine (Kierstead et al 1983 [compound 4D]), which was reported in initial screening studies to have a selectivity ratio of more than 10 000.

Materials and methods

Common reagents were from Sigma Chemical Co. (St. Louis, MO). ICI 118,551 [erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol] and the erythro-desmethyl analogue of ICI 118,551 were obtained from Imperial Chemical Industries (Wilmslow, Cheshire, UK). The 7-ethylindan analogue of ICI 118,551; the didesmethyl t-butyl analogue [erythro-DL-1-(indan-4-yloxy)-3-t-butylaminopropan-2-ol]; and the binary aryloxypropanolamine, 1,1′-((2-hydroxy-1,3-propanediyl)bis(oxy - 2,1 - phenyleneoxy))bis(3 - ((1 - methyl ethyl) amino)-2(*S*)-propanol) 2HCl (4d), were kindly synthesized by Dr John Baldwin and colleagues at Merck and Co. (West Point, PA). The structures of these compounds are shown in Fig. 1.

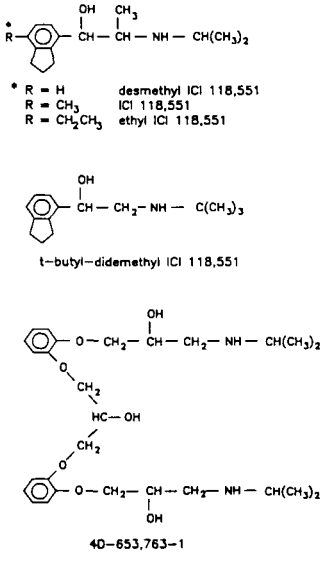

FIG. 1. Structures of compounds used in the present experiments.

Rabbit eyes and heart ventricle were from New Zealand albino males, 3–6 months old. Ciliary process villae were dissected, as previously described, from a posterior approach (Nathanson 1981b, 1985b), and homogenized (10 mg mL^{-1}) by hand in an all-glass homogenizer in 6 mM Tris maleate buffer, pH 7·4. Heart tissue was minced and then homogenized (20–50 mg mL^{-1}) as above. Previous studies have established that activity measurements of β-adrenergic-stimulated adenylate cyclase reflect the ability of agonists or antagonists to activate or block the β-adrenoceptor (Perkins et al 1982). Therefore, in the present experiments, inhibition (by various concentrations of an antagonist) of isoprenaline-stimulated activation of ciliary process and cardiac adenylate cyclase was used to determine the inhibitory constants (K_i) described below. Adenylate cyclase activity was measured as described previously (Nathanson 1980) using the following reaction mixture (0·3 mL volume) mM: Tris maleate 80 (pH 7·4), theophylline 10, ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA) 0·5, $MgCl_2$ 8, GTP 0·03, ATP 2, tissue homogenate (0·7–3 mg wet weight), plus or minus test substances as indicated. To allow for possible differences in membrane lipid solubility of antagonists, drugs were preincubated (10 min at 0°C) with tissue and buffer, before the addition of isoprenaline and the subsequent initiation of the

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adenylate cyclase reaction with ATP and GTP. Cyclic AMP produced during the 4 min linear reaction (30°C; terminated by boiling) was measured by protein binding assay (Brown et al 1972), and protein concentration determined by the method of Lowry et al (1951). Basal adenylate cyclase activity in ciliary process was, typically, 10–20 pmol (mg protein)$^{-1}$ min^{-1} and, in the heart, 30–40 pmol (mg protein)$^{-1}$ min^{-1}.

Activation constants (K_a) and IC50 values were determined using 12–16 data points per dose-response curve. For the inhibition curves, various concentrations of antagonist were tested against a fixed concentration of isoprenaline (usually 10^{-5} M). In various experiments, 10^{-5} M isoprenaline stimulated basal activity from 90–190% in ciliary process and from 50–70% in heart ventricle. Stimulation in the presence of isoprenaline plus antagonist was calculated as the increase over that seen in the presence of antagonist alone. This procedure controlled for a small inhibitory effect on basal adenylate cyclase activity seen when higher concentrations ($> 10^{-5}$ M) of antagonists were used. In the ciliary process, this inhibition of basal activity was usually less than 15%. In the heart, it varied from 10–40%. K_i values for the various antagonists were calculated from the equation (Cheng & Prusoff 1973), $K_i = (IC50)/(1 + S/K_a)$; where IC50 = the concentration of antagonist necessary to give 50% inhibition of activitity in the presence of isoprenaline, S = the concentration of isoprenaline present, and K_a = the concentration of isoprenaline necessary for half-maximal activation of adenylate cyclase activity in the particular tissue used (ciliary process or heart).

The relative selectivity of a particular compound for inhibiting β-adrenoceptors in ciliary process versus heart was defined through a potency ratio, which was calculated by dividing the K_i obtained in the heart by the K_i obtained in the ciliary process. A ratio greater than 1 indicates relative selectivity for ciliary process (oculoselective), while a ratio less than 1 indicates β_1 selectivity. In previous studies, we have reported potency ratios ranging from 0·32 for practolol to 510 for ICI 118,551 (Nathanson 1985b).

Results

Fig. 2 shows the effects of ICI 118,551 (a methyl derivative) and its ethyl and desmethyl derivatives in inhibiting isoprenaline stimulation in the ciliary process and heart. All three compounds showed oculoselectivity and therefore the curves for inhibiting ciliary process adenylate cyclase appear to the left of those for inhibiting adenylate cyclase in the heart. In the ciliary process, the compounds differed substantially in potency: the rank order potency among the three derivatives was ethyl > methyl > desmethyl. In the heart, potencies differed by a smaller degree, with a rank order of desmethyl > ethyl > methyl. Because of these differences in ciliary process and heart, the ethyl derivative had the greatest degree of oculoselectivity, as shown by the fact that the inhibitory curves for this derivative in the two tissues displayed the largest degree of separation. The desmethyl derivative had the smallest degree of oculoselectivity and, accordingly, the separation between ciliary process and heart was much less. For all experiments, Table 1 shows the calculated K_i values for the three compounds in blocking β-adrenoceptors in the ciliary process and heart. This Table also shows the calculated potency ratios of the compounds in discriminating between the two tissues.

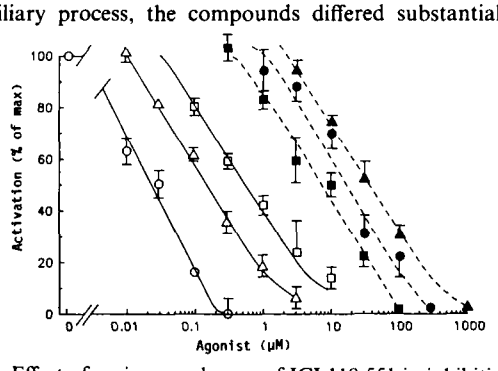

FIG. 2. Effect of various analogues of ICI 118,551 in inhibiting the isoprenaline-stimulated activation of ciliary process (open symbols) and cardiac ventricle (closed symbols) adenylate cyclase. Values shown here and in Fig. 3 are the mean (± range) for replicate samples, each assayed in duplicate (i.e., a total of 4 cAMP determinations per data point). Curves are from a particular experiment and are typical of those seen in other experiments. ●,○ = ethyl derivative; ▲,△ = methyl derivative (ICI 118,551); ■,□ = desmethyl derivative.

Fig. 3 shows the effects of the didesmethyl t-butyl derivative of ICI 118,551. The degree of oculoselectivity for the didesmethyl derivative is similar to that for the desmethyl analogue, although in both tissues the absolute potency of the former derivative is greater (Table 1). Fig. 3 also shows the moderate degree of oculoselectivity of the binary aryloxypropanolamine, 4d, with a ciliary process/cardiac potency ratio of 11.

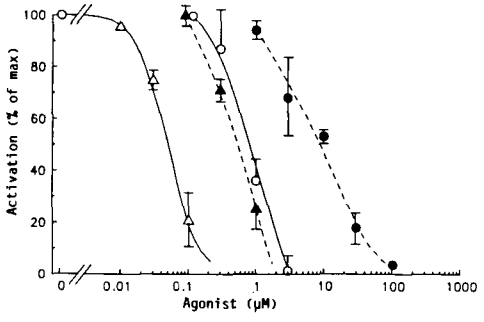

FIG. 3. Effect of didemethyl-t-butyl ICI 118,551 (▲,△) and 4D (●,○) on rabbit ciliary process (open symbols) and cardiac ventricle (closed symbols) isoprenaline-stimulated activated adenylate cyclase.

Table 1. Potency and selectivity of ICI 118,551 derivatives and 4D in blocking the activation of isoprenaline-stimulated adenylate cyclase in heart and ciliary process (CP).

Antagonist	K_i (micromolar) CP	K_i (micromolar) Heart	Potency ratio Heart/CP
Ethyl derivative	0·00072	0·52	720
ICI 118,551 (methyl)	0·0043	1·9	440
Desmethyl derivative	0·023	0·21	9·3
Didesmethyl-t-butyl	0·0017	0·014	8·2
4D	0·025	0·28	11

Discussion

These data show that all five β-adrenoceptor antagonists evaluated are oculoselective, with eye/heart potency ratios ranging from a modest 8·2 for the t-butyl-didesmethyl derivative of ICI 118,551 to a substantial 720 for the 7-ethyl derivative. This latter value represents the highest oculoselectivity ratio (ciliary process versus heart) yet reported for a beta-blocker.

Bilski et al (1979) originally reported that ICI 118,551 had a β_2 selectivity ratio of about 125. In subsequent physiological experiments comparing inhibition of β-adrenergic-induced atrial chronotropism and tracheal relaxation, ICI 118,551 has been reported to have selectivity ratios ranging from 3 to 54 (Schmitt et al 1984; O'Donnell & Wanstall, 1980) with a recent extensive study by Lemoine et al (1985) yielding a value of about 300. Previous biochemical studies comparing inhibi-

tion of isoprenaline stimulated adenylate cyclase in ciliary process versus cardiac ventricle have shown a somewhat greater degree of selectivity, with selectivity ratios ranging from 480 to 510 (Nathanson 1984, 1985b). There have been no previous physiological or biochemical studies evaluating the selectivity of the ethyl derivative, which in the present experiments was more selective than ICI 118,551 itself.

A comparison of the data for the desmethyl versus methyl (ICI 118,551) versus ethyl derivative indicates that progressive addition of, first, a methyl and then an ethyl group at position 7 of the indan moiety results in a corresponding increase in oculoselectivity. This increase is due primarily to an increase in potency at the ciliary process β_2-receptor. For the desmethyl derivative, its somewhat higher potency at the cardiac β-adrenoceptor serves to further decrease its oculoselectivity. In future studies, it will be of interest to determine if addition of larger alkyl groups, such as n-propyl, to the 7-position may further increase oculoselectivity.

The didesmethyl derivative of ICI 118,551 differs from the above compounds by having two alterations in the alkyl side chain, and for this reason it is not appropriate to compare it with the above series. However, it is of interest that loss of the α-methyl group results in a substantial (about 15-fold) increase in potency at both receptors. Previously, we have observed a similar change at the cardiac receptor, as noted by the comparison of α-methyl-propranolol with propranolol, which results in a 14-fold increase in potency (Nathanson 1985b).

The binary β-adrenoceptor blocker, 4D, was originally synthesized as one of a group of symmetrical agents consisting of two (*S*)-(phenyloxy)propranolamine groups coupled through alkylenedioxy or poly(oxyethylenedioxy) linking units of varying lengths (Kierstead et al 1983). Although agents with β_1 selectivity were being sought, 4d was noted to have considerable β_2 selectivity (15 000:1) when screened in-vitro using intact guinea-pig atria and trachea. Affinity constants were reported to be 0·65 μM for inhibiting isoprenaline-stimulated chronotropy and 0·044 nM for inhibiting isoprenaline-induced tracheal relaxation. In the present experiments, the value obtained for inhibition of cardiac β-adrenergic-stimulated adenylate cyclase (0·28 μM) is similar to that obtained by Kierstead et al (1983) in isolated atria. However, our value for inhibition of the β_2-stimulated adenylate cyclase in the ciliary process is 0·025 μM or about 500-fold greater than the value obtained by Kierstead et al using intact trachea. Although we have no definitive explanation for this difference, it is of interest that, when Kierstead et al carried out additional evaluation of beta-selectivity of 4D in-vivo, a selectivity ratio of 22 was obtained, much closer to our value of 11 than to the value of 15 000 which these workers first obtained using isolated intact tissue. It appears that further investigation will be necessary before the reportedly enormous β_2 selectivity of this compound can be confirmed.

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