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The Receptor Binding Profile of the New Antihypertensive Agent Nebivolol and Its Stereoisomers Compared With Various β-Adrenergic Blockers

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SUMMARY

Nebivolol [the (*S,R,R,R*)- + (*R,S,S,S*)-racemic mixture], the 10 stereoisomers, and known β-adrenergic blockers were investigated *in vitro* for binding to β_1- and β_2-adrenergic receptor sites and various neurotransmitter, peptide, and ion channel binding sites and for inhibition of neurotransmitter uptake. Selective labeling of β_1- and β_2-adrenergic receptor sites in rabbit and rat lung, respectively, was obtained with [^{3}H]CGP-12177 and [^{3}H] dihydroalprenolol in the presence of an appropriate concentration of the selective β_2-adrenergic blocker ICI 118-551 or the selective β_1-adrenergic blocker CGP 20712-A. Nebivolol revealed high affinity and selectivity for β_1-adrenergic receptor sites in the rabbit lung membrane preparation (K_i value = 0.9 nM and β_2/β_1 ratio = 50). The drug dissociated slowly from these receptor sites. The activity resided in the (*S,R,R,R*)-enantiomer (R 67 138); the (*R,S,S,S*)-enantiomer (R 67 145) revealed 175 times lower β_1-adrenergic binding affinity. Within the series of stereoisomers, nebivolol and R 67 138 showed the best combination of high affinity and selectivity. Among the reference compounds, only CGP 20712-A shared these properties. Nebivolol bound to S_{1A} binding sites with a K_i value of 20 nM. The stereospecific requirements for interaction with these sites were different from those for the β_1-adrenergic receptor site. S_{1A} binding site affinity was also observed with the potent but nonselective β-adrenergic blockers carvedilol, pindolol, and propranolol. In the various other investigated radioligand binding and neurotransmitter uptake assays, nebivolol and its stereoisomers showed activity only at micromolar concentrations or were inactive. Clinical studies have shown an interesting hemodynamic profile of nebivolol, offsetting the negative effects on left ventricular performance generally observed with classical β-adrenergic blockers. Several hypotheses regarding the mechanism of action of nebivolol are summarized.

Nebivolol (R 65 824) (nebivolol-hydrochloride is R 67 555), (±)-[*R**[*S**[*S**-(S*)]]]-α,α′-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2*H*-1-benzopyran-2-methanol], is a pseudosymmetrical molecule with four asymmetric carbon atoms (Fig. 1). Ten stereoisomers, comprising four enantiomeric pairs and two mesoforms, were synthesized and isolated.[1] Nebivolol, the racemic mixture of the (*S,R,R,R*)- and (*R,S,S,S*)-enantiomers, is being investigated as a new antihypertensive agent. In clinical studies with hypertensive patients, nebivolol was found to reduce heart rate and blood pressure but it also improved left ventricular function (1–3). In animal pharmacological studies, immediate reduction in blood pressure was observed with nebivolol, after its administration to conscious spontaneously hypertensive rats. No such effect was observed with known β-adrenergic blockers such as atenolol, propranolol, or pindolol. A further unusual feature observed at low doses of nebivolol was its apparent lack of negative cardiac inotropic effect in anesthetized dogs, in comparison with propranolol. Nebivolol reduced systemic vascular resistance and increased cardiac output and stroke volume. At equivalent doses, propranolol reduced cardiac output and stroke volume. Pharmacological investigations using isolated tissues have revealed a potent antagonism by nebivolol of isoprenaline-induced effects mediated by β_1-adrenergic receptors in the guinea pig atrium. However, the compound was 300-fold less active in antagonizing β_2-adrenergic receptor-mediated effects in the guinea pig trachea. A selective action of nebivolol at β_1-adrenergic receptors *in vivo* is apparent from the greater inhibition of isoprenaline-induced changes of left ventricular contractility mediated by cardiac β_1-adrenergic receptors, as compared with the reduction in diastolic blood pressure (vascular β_2-adrenergic receptors) in dogs (3).

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[1] Van Lommen *et al.*, manuscript in preparation.

ABBREVIATIONS: CGP20712-A, (±)-(2-hydroxy-5-[2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1*H*-imidazole-2-yl)phenoxy)propyl)amino)ethoxy]-benzamide monomethane sulfonate; [^{3}H]CGP-12177, (±)-[^{3}H]4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on hydrochloride; ICI-118551, erythro-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol.

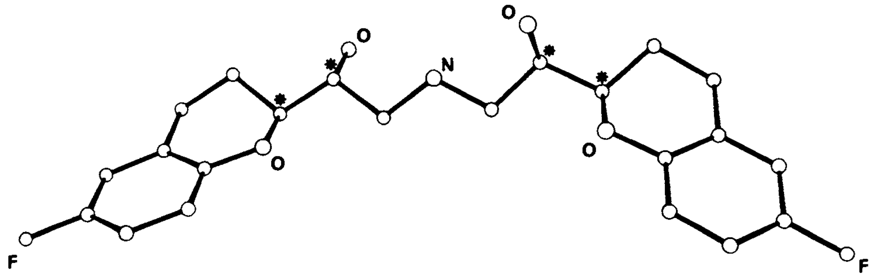

Fig. 1. Structure of nebivolol with indication (*) of the four asymmetric carbon atoms.

In this study, the receptor binding and neurotransmitter uptake inhibition properties of nebivolol were investigated. Specific radioligand binding models have been developed for selective labeling of β_1- and β_2-adrenergic receptor sites. This was achieved using (i) selective tissues, i.e., rabbit and rat lung for β_1- and β_2-adrenergic receptor sites, respectively, (ii) the selective β_1-adrenergic receptor blocker CGP 20712-A (4) and β_2-adrenergic receptor blocker ICI 118-551 (5, 6), and (iii) [^{3}H]CGP-12177 and [^{3}H]dihydroalprenolol as radioligands. The stereoselectivity of the β-adrenergic receptor interaction with the nebivolol stereoisomers was investigated. The dissociation rate of unlabeled drugs from the β_1- and β_2-adrenergic receptors was measured by modification of a previously described tissue-adsorbed-to-filter method (7, 8). The interaction of nebivolol stereoisomers with various neurotransmitter receptors, ion channels, and peptide binding sites was investigated. The potency of these compounds to inhibit monoamine uptake in rat brain synaptosomes was tested. The β-adrenergic selectivity and profile of nebivolol and its stereoisomers were compared with those of known β-adrenergic blockers. The biochemical profile of nebivolol is discussed in light of the reported pharmacological properties and findings in clinical studies.

Materials and Methods

Tissue preparation. Lungs from male New Zealand rabbits (~2 kg) and female rats (~150 g) were dissected and transferred in 0.9% NaCl. Tissue was homogenized in 10 volumes (volumes per wet weight tissue, v/w) of buffer (0.25 M sucrose, 0.15 M $NaClO_4 \cdot H_2O$, 5 mM EDTA, and 25 mM imidazol, pH 7.4) with a Polytron mixer (3 × 10 sec, 1500 rpm). The homogenate was centrifuged at 830 × *g* for 10 min to precipitate cell nuclei and debris. The pellet was rehomogenized and similarly centrifuged. The supernatants were combined, and filtered over cheesecloth, and further diluted up to 40 volumes per wet weight with 50 mM Tris·HCl, pH 7.7. This suspension was centrifuged at 23,600 × *g* for 20 min, to precipitate the cell membranes. The pellet was washed twice by suspension in Tris·HCl buffer and centrifuged. The final pellet was homogenized with a Duall homogenizer in 10 volumes of 50 mM Tris·HCl, pH 8. During the entire preparation procedure the tissue suspension was kept at 0–4°. The membrane preparation was distributed into aliquots and stored at −70°. For binding assays, the membrane preparation was diluted to 100 volumes (v/w) with 50 mM Tris·HCl, pH 8.

Binding assays to β_1- and β_2-adrenergic receptor sites. Incubation mixtures were composed of 2 ml of tissue preparation, 0.1 ml of [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol, with or without drug for binding site occlusion, and 0.1 ml of solvent (10% ethanol), drug for inhibition, or drug for determination of nonspecific binding. Samples were mixed and incubated for 15 min at 37°. The reactions were stopped by adding 5 ml of ice-cold Tris·HCl buffer, pH 8.0, and rapid filtration over Whatman GF/B glass fiber filters under vacuum. The filters were rapidly rinsed twice with 5 ml of ice-cold Tris·HCl buffer, pH 8.0. Filters were placed in scintillation vials and radioactivity was extracted by vigorous shaking in 8 ml of Instagel II (Packard, Warrenville). The radioactivity was counted in a Packard Tri-carb 4530 liquid scintillation counter.

To measure binding to β_1-adrenergic binding sites in rabbit lung membranes, 10 nM ICI 118-551 was added with [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol for occlusion of β_2-adrenergic binding sites. Nonspecific binding was measured in the additional presence of 1 μM CGP 20712-A. To measure binding to β_2-adrenergic binding sites in rat lung membranes, 300 nM CGP 20712-A was added with [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol for occlusion of β_1-adrenergic binding sites. Nonspecific binding was defined in the additional presence of 1 μM ICI 118-551.

To measure potencies of drugs for inhibition of binding, 1 nM [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol was used. The drugs were added to the incubation mixtures in at least six concentrations, spanning 4 orders of magnitude. The specific [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol binding in the presence of drug was calculated as the percentage of total [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol binding and plotted versus the log of the drug concentration. IC_{50} values (concentration inhibiting 50% of specific [^{3}H]ligand binding) were derived graphically. K_i values were calculated according to the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + C/K_d)$ with C being the concentration and K_d the equilibrium dissociation constant of the [^{3}H]ligand (9).

For saturation binding curves, [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol was used at concentrations between 0.05 and 1 nM. K_d and B_{max} values were derived from Scatchard plots. Linear regression lines were calculated by the method of least squares.

Measurement of β_1- and β_2-adrenergic receptor dissociation rates. The *in vitro* dissociation rates of the unlabeled drugs from the β_1- and β_2-adrenergic receptor sites were measured using a tissue-absorbed-to-filter method as previously described (7, 8), with modifications. A tissue membrane preparation (see above), saturated with drug during preincubation with a concentration of 10 × IC_{50} value, was adsorbed to Whatman GF/B glass fiber filters positioned on the filtration apparatus. The drug-loaded tissues, adsorbed to the filters, were rinsed with warm buffer for various time periods. At the end of the rinsing period, the tissue, adsorbed to the filter, was incubated with a sample of [^{3}H]CGP-12177 to quantify free receptors. Calculation of the half-time of dissociation of the unlabeled drug was as previously described (7).

Radioligand receptor binding and neurotransmitter uptake assays. Radioligand binding assays were performed using rat or guinea pig brain membrane preparations (10). For neurotransmitter uptake, a crude synaptosomal fraction from rat brain regions was used (10). The assay conditions for serotonin S_2, serotonin S_{1A}, dopamine D_2, dopamine D_1, α_1-adrenergic, α_2-adrenergic, histamine H_1, cholinergic-muscarinic, μ opiate, benzodiazepine, dihydropyridine, biogenic amine, and metabolite release, substance P and neurotensin receptor binding, and serotonin, noradrenaline, dopamine and γ-aminobutyric acid uptake were as previously described.[2] Binding to the veratridine site of the

[2] Leysen, J. E., W. Gommeren, A. Eens, D. de Chaffoy de Courcelles, J. C. Stoof, and P. A. J. Janssen. Biochemical profile of risperidone, a new antipsychotic. *J. Pharmacol. Exp. Ther.* **247**:661–670 (1988).

Na^+ channel was measured with tetraphenylphosphonium ions as previously described (11).

Materials. (-)-[^{3}H]CGP-12177 (34 Ci/mmol) was from Amersham and *l*[propyl-1,2,3-[^{3}H]dihydroalprenolol-HCl (48 Ci/mmol) was obtained from New England Nuclear (Dreieich, Germany). Nebivolol and stereoisomers were original substances from Janssen Pharmaceutica (Beerse, Belgium). Other drugs were generously supplied by the companies of origin.

Results

Development of receptor binding models for selective labeling of β_1- and β_2-adrenergic receptors. Inhibition of [^{3}H]CGP-12177 binding by the selective β_1- and β_2-adrenergic blockers CGP 20712-A and ICI 118-551, respectively, was measured in rabbit and rat lung membrane preparations; inhibition curves are shown in Fig. 2. In rabbit lung, CGP 20712-A showed a monophasic inhibition curve and inhibited 80% of total [^{3}H]CGP-12177 binding. The inhibition curve of ICI 188-551 was biphasic; it was noted that less than 15% of total bound [^{3}H]CGP-12177 was inhibited in the nanomolar range. In contrast, ICI 118-551 inhibited, at nanomolar concentrations, 80% of total [^{3}H]CGP-12177 binding in the rat lung membrane preparation. In this preparation, CGP 20712-A showed a biphasic inhibition curve; only 25% of the total [^{3}H]CGP-12177 binding was inhibited in the nanomolar range. These findings indicated that rabbit and rat lung membrane preparations were enriched in β_1- and β_2-adrenergic receptor sites, respectively. In subsequent experiments, the minor population of β_2- and β_1-adrenergic receptor sites in rabbit and rat lung was occluded by addition of 10 nM ICI 118-551 and 300 nM CGP 20712-A to rabbit and rat lung membrane preparations, respectively. Figs. 3 and 4 show the saturation binding curves of [^{3}H]CGP-12177 in rabbit and rat lung membrane preparations under such conditions. Scatchard analysis revealed a single population of binding sites in each of the tissues, representing β_1-adrenergic receptor sites in the rabbit lung and β_2-adrenergic receptors sites in the rat lung. Similar findings were obtained with [^{3}H] dihydroalprenolol. K_d and B_{max} values for [^{3}H]CGP-12177 and [^{3}H]dihydroalprenolol binding are summarized in Table 1. [^{3}H]

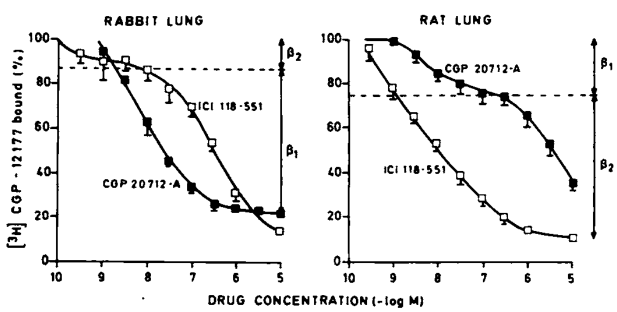

Fig. 2. Inhibition of total [^{3}H]CGP-12177 binding to rabbit and rat lung membrane preparations by CGP 20712-A and ICI 118-551. Binding was performed with 1 nM [^{3}H]CGP-12177 as described in Materials and Methods. In rabbit lung, total and nonspecific binding (in the presence of 1 μM CGP 20712-A) represent 16,690 ± 2,950 dpm and 3,713 ± 623 dpm, respectively. Rat lung, total and nonspecific binding (in the presence of 1 μM ICI 118-551) represent 17,511 ± 2,091 dpm and 2,547 ± 387 dpm, respectively. [^{3}H]CGP-12177 binding is expressed as percentage of total binding in the absence of unlabeled drugs. β_1, β_1-adrenergic receptor site; β_2, β_2-adrenergic receptor site. *Curves* were constructed using mean values ± standard error of three separate experiments performed in duplicate.

CGP-12177 bound with high affinity to β_1- and β_2-adrenergic receptor sites, the affinity for the β_1-adrenergic receptor being slightly higher. [^{3}H]Dihydroalprenolol bound with higher affinity to β_2- than β_1-adrenergic receptor sites. Its β_2-adrenergic receptor affinity was similar to that of [^{3}H]CGP-12177. The density of β_1-adrenergic receptor sites in the rabbit lung membrane preparation was equal to the density of β_2-adrenergic receptor sites in the rat lung membrane preparation. The receptor densities obtained with [^{3}H]dihydroalprenolol binding were in the same range.

Interaction of nebivolol, its stereoisomers, and various β-adrenergic blockers with β_1- and β_2-adrenergic receptor sites. Fig. 5 shows the inhibition curves of nebivolol, its *d*-enantiomer R 67 138 (*S,R,R,R*), and its *l*-enantiomer R 67 145 (*R,S,S,S*) on [^{3}H]CGP-12177 binding to β_1- and β_2-adrenergic receptor sites in rabbit and rat lung membrane preparations, respectively. Nebivolol and R 67 138 were as potent as CGP 20712-A in the inhibition of [^{3}H]CGP-12177 binding to rabbit lung membrane preparation. R 67 145 was 100 times less active than R 67 138. In contrast, [^{3}H]CGP-12177 binding to rat lung membrane preparation was only weakly inhibited by nebivolol and its two enantiomers. Nebivolol and R 67 138 were 100 times less potent than ICI 118-551 whereas R 67 145 was still 10 times less active. The eight remaining stereoisomers of nebivolol were similarly investigated; the binding affinities for β_1- and β_2-adrenergic receptor sites measured with [^{3}H]CGP-12177 and [^{3}H]dihydroalprenolol are summarized in Table 2. Nebivolol and R 67 138 showed the highest affinity for β_1-adrenergic receptors and they revealed a β_1/β_2 receptor selectivity of 40- to 50-fold. The most pronounced β_1-adrenergic selectivity (70–100-fold) was found with R 74 718 (*R,R,R,R*), but its β_1-adrenergic affinity was 12-fold less than that of R 67 138.

The β-adrenergic receptor binding affinity and selectivity of various known β-adrenergic blockers is shown in Table 3. Carvedilol, pindolol, and propranolol potently bound to β_1- and β_2-adrenergic receptor sites and lacked selectivity. Levantolol and labetolol were less potent and also nonselective. CGP 20712-A was potent and highly selective for β_1-adrenergic receptor sites whereas ICI 118-551 was a selective compound for β_2-adrenergic receptor sites. Atenolol showed low affinity for β_1- and β_2-adrenergic receptor sites and only moderate selectivity.

The dissociation rates of the compounds from the β_1- and β_2-adrenergic receptor sites were measured using the tissue-adsorbed-to-filter technique. The half-times of dissociation are presented in Table 4. Labetolol, pindolol, propranolol, and levantolol dissociated within a few minutes from both the β_1- and the β_2-adrenergic receptor sites. By contrast, nebivolol, R 67 138 (*S,R,R,R*), ICI 118-551, and carvedilol dissociated slowly from the β_1- and β_2-adrenergic receptor site.

Interaction of nebivolol, its stereoisomers, and various β-adrenergic blockers with neurotransmitter receptors, ion channels, and peptide binding sites, and neurotransmitter uptake. The binding affinity *in vitro* of the nebivolol stereoisomers was measured in radioligand binding assays for neurotransmitter receptor sites, ion channels and peptide binding sites. The binding affinities of the stereoisomers expressed as $-\log IC_{50}$ values and K_i values are shown in Table 5. The potencies of the drugs ($-\log IC_{50}$) to inhibit the uptake of serotonin, norepinephrine, dopamine, and γ-aminobutyric acid

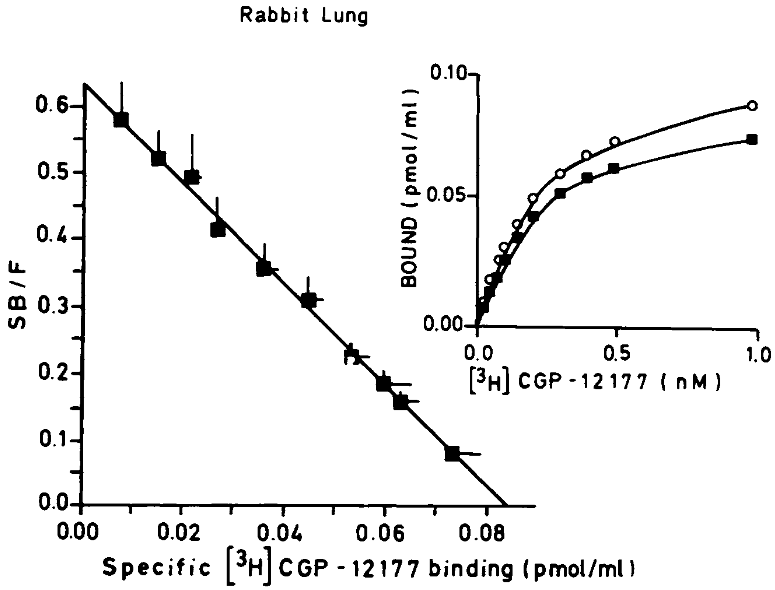

Fig. 3. Saturation binding curve (*inset*) and Scatchard plot of [^{3}H]CGP-12177 binding to β_1-adrenergic receptor sites in rabbit lung membrane preparation. Binding was carried out in the presence of 10 nM ICI 118-551 to block β_2-adrenergic receptor sites. Nonspecific binding was defined in the presence of 1 μM CGP 20712-A (○, total binding; ■, specific binding). *Curves* were constructed using mean values of binding data from four separate experiments. *SB*, specific [^{3}H]CGP-12177 binding, total bound [^{3}H]CGP-12177 in the presence of 10 nM ICI 118-551 minus nonspecifically bound. *F*, free [^{3}H]CGP-12177 concentration, added concentration of [^{3}H]CGP-12177 minus the total concentration bound. K_d value was given by the reciprocal value of the slope of the lines. B_{max} value was given by the intersection point with the *abscissa* (in pmol/ml). *Lines* were calculated using the method of least squares. Values are presented in Table 1.

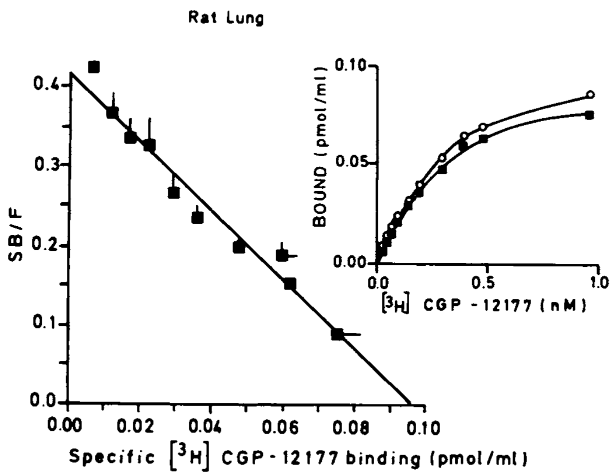

Fig. 4. Saturation binding curve (*inset*) and Scatchard plot of [^{3}H]CGP-12177 binding to β_2-adrenergic receptor sites in rat lung membrane preparation. Binding was carried out as described in the legend to Fig. 3 except that 300 nM CGP 20712-A was used instead of 10 nM ICI 118-551, to block β_1-adrenergic receptor sites. ○, Total binding; ■, specific binding. Derived K_d and B_{max} values are presented in Table 1.

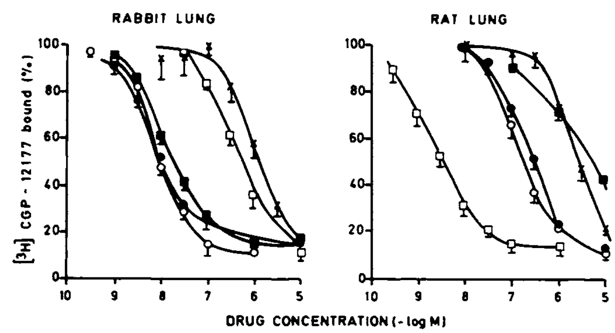

Fig. 5. Inhibition of [^{3}H]CGP-12177 binding to β_1- and β_2-adrenergic receptor sites in rabbit and rat lung membrane preparation, respectively, by nebivolol (○), R 67 138 (●), R 67 145 (×), CGP 20712-A (■), and ICI 118-551 (□). Rabbit lung binding was in the presence of 10 nM ICI 118-551; total binding, 13874 ± 1327 dpm; and nonspecific binding, 2475 ± 103 dpm. Rat lung binding was in the presence of 300 nM CGP 20712-A; total binding, 13384 ± 2199 dpm; and nonspecific binding, 1841 ± 228 dpm. [^{3}H]CGP-12177 binding is expressed as percentage of total binding in the presence of 10 nM ICI 118-551 and 300 nM CGP 20712-A for rabbit and rat lung, respectively. *Curves* were constructed using mean ± standard error values of three separate experiments in duplicate.

TABLE 1

***K_d* and *B_{max}* values of [^{3}H]CGP-12271 and [^{3}H]dihydroalprenolol binding to β_1- and β_2-adrenergic receptor sites in rabbit and rat lung membrane preparation.**

K_d and B_{max} values are the means ± standard error of values obtained in four separate experiments.

	Rabbit lung β_1		Rat lung β_2	
	K_d	B_{max}	K_d	B_{max}
	nM	*fmol/mg of tissue*	*nM*	*fmol/mg of tissue*
[^{3}H]CGP-12177	0.14 ± 0.01	9.3 ± 0.7	0.24 ± 0.04	10.7 ± 0.7
[^{3}H]Dihydroalprenolol	0.89 ± 0.23	5.1 ± 1.3	0.21 ± 0.03	7.7 ± 0.4

in crude synaptosomal preparations are shown in Table 6. Several of the nebivolol stereoisomers bound to S_{1A} binding sites labeled with [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin; nebivolol, R 65 825, R 67 138, R 65 260, R 74 716, R 74 829, and R 67 142 showed K_i values between 20 and 40 nM. In the various other investigated radioligand binding and neurotransmitter uptake assays, nebivolol and its stereoisomers showed only activity at micromolar concentrations or were inactive.

In order to better visualize the profile of the nebivolol stereoisomers, pie charts have been constructed for nebivolol, R 67 138, and R 67 145 using the reciprocal of the K_i values for receptor binding and IC_{50} values for inhibition of monoamine uptake (Fig. 6). The pie chart shows the relative contribution of each activity in the sum of activities of the drug presented in Tables 2, 5, and 6. For nebivolol, β_1-adrenergic receptor binding accounts for 93%, β_2-adrenergic receptor binding for

TABLE 2

Binding affinity of nebivolol stereoisomers for β_1- and β_2-adrenergic receptor sites measured with two ligands

a, −log IC_{50} (M), mean value ± standard deviations. Numbers in parentheses, number of experiments. b, K_i values (nM). Binding was performed as described in Materials and Methods in the presence of 300 nM ICI 118-551 with rabbit lung membrane preparation and 10 nM CGP 20717-A with rat lung membrane preparation to measure β_1- and β_2-adrenergic receptor sites, respectively.

R-number, Configuration		[³H]CGP-12177 binding (1 nM)			[³H]Dihydroalprenolol binding (1 nM)		
		Rabbit lung β_1	Rat lung β_2	Ratio β_2/β_1	Rabbit lung β_1	Rat lung β_2	Ratio β_2/β_1
Nebivolol	a.	8.13 ± 0.05 (3)	6.6 (2)		8.72 ± 0.09 (4)	6.57 ± 0.05 (4)	
S,R,R,R + *R,S,S,S*	b.	0.88	48	55	0.91	44	48
R 65 825	a.	7.53 ± 0.05 (3)	6.15 ± 0.07 (3)		7.92 ± 0.09 (4)	6.13 ± 0.11 (3)	
S,R,S,S + *R,S,R,R*	b.	3.5	144	41	5.7	281	49
R 67 138	a.	8.17 ± 0.06 (3)	6.76 ± 0.05 (3)		8.95 ± 0.1 (4)	6.96 ± 0.15 (3)	
S,R,R,R	b.	0.8	34	42	0.54	19	35
R 67 145	a.	5.93 ± 0.11 (3)	5.66 ± 0.05 (2)		6.53 ± 0.05 (3)	5.66 ± 0.11 (3)	
R,S,S,S	b.	140	423	3	138	367	2.6
R 65 260	a.	7.65 ± 0.07 (2)	6.70 ± 0.14 (2)		8.2 (2)	6.40 ± 0.14 (2)	
S,R,R,S	b.	2.7	39	15	3	68	23
R 74 716	a.	6.15 ± 0.07 (2)	5.65 ± 0.2 (2)		6.8 (2)	5.69 ± 0.07 (2)	
R,S,S,R	b.	84	433	5.15	75	375	5
R 74 829	a.	6.5 (2)	6.20 ± 0.14 (2)		7.05 ± 0.07 (2)	6.15 ± 0.2 (2)	
S,R,S,R	b.	38	122	3.2	43	125	2.9
R 74 714	a.	6.60 ± 0.14 (2)	5.95 ± 0.07 (2)		7.25 ± 0.07 (2)	6.0 (2)	
S,R,S,S	b.	30	217	7.2	27	166	6.1
R 67 142	a.	7.5 (2)	6.10 ± 0.14 (2)		7.90 ± 0.14 (2)	6.0 (2)	
R,S,R,R	b.	3.8	153	40	6	166	28
R 74 721	a.	5.95 ± 0.07 (2)	5.15 ± 0.07 (2)		6.40 ± 0.07 (2)	5.30 ± 0.14 (2)	
R,R,S,S	b.	133	1370	10	193	857	4.4
R 74 723	a.	5.1 (2)	5.30 ± 0.14 (2)		5.40 ± 0.14 (2)	5.19 ± 0.07 (2)	
S,S,S,S	b.	945	971	1	1935	1187	0.6
R 74 718	a.	7.05 ± 0.07 (2)	5.2 (2)		7.65 ± 0.07 (2)	5.35 ± 0.07 (2)	
R,R,R,R	b.	11	1222	111	11	744	68

TABLE 3

Binding affinity of various β-adrenergic blockers for β_1- and β_2-adrenergic receptor sites measured with two ligands

a, −log IC_{50} (M), mean value ± standard deviation. Numbers in parentheses, number of experiments. b. K_i value (nM). Binding was performed as described in the legend to Table 2.

		[³H]CGP-12177 binding (1 nM)			[³H]Dihydroalprenolol binding (1 nM)		
		Rabbit lung β_1	Rat lung β_2	Ratio β_2/β_1	Rabbit lung β_1	Rat lung β_2	Ratio β_2/β_1
CGP 20712-A	a.	7.86 ± 0.05 (3)	5.2		8.35 (2)	5.3 (2)	
	b.	1.6	1222	763	2.1	835	397
Atenolol	a.	5.65 ± 0.07 (2)	4.8 (2)		6.25 ± 0.07 (2)	4.75 ± 0.07 (2)	11
	b.	396	7493	19	266	2960	
Levantolol	a.	6.90 ± 0.07 (2)	6.60 ± 0.14 (2)		7.5 (2)	6.50 ± 0.14 (2)	
	b.	15	49	3.2	15	53	3.5
Labetolol	a.	6.7 (2)	7.0 (2)		7.40 ± 0.14 (2)	6.79 ± 0.07 (2)	
	b.	24	19	0.79	19	30	1.58
Carvedilol	a.	8.65 ± 0.07 (2)	9.0 (2)		9.02 ± 0.09 (4)	8.81 ± 0.02 (3)	
	b.	0.24	0.19	0.79	0.43	0.25	0.58
Pindolol	a.	8.13 ± 0.11 (3)	8.30 ± 0.1 (3)		8.66 ± 0.11 (3)	8.33 ± 0.15 (3)	
	b.	1.4	1.0	0.7	1.0	0.8	0.8
Propranolol	a.	7.83 ± 0.05 (3)	8.5 (2)		8.6 (2)	8.75 (2)	
	b.	2.8	0.62	0.22	1.2	0.29	0.24
ICI 118-551	a.	6.60 ± 0.14 (2)	8.60 ± 0.07 (2)		7.12 ± 0.09 (4)	8.43 ± 0.10 (3)	
	b.	49	0.49	0.01	36	0.62	0.02

1.7%, and S_{1A} binding site binding for 4.1%. The contribution of the other activities listed in Tables 5 and 6 is negligible. The relative contribution of each activity of R 67 138 (*S,R,R,R*) was similar. However, the chart of R 67 145, the (*R,S,S,S*)-enantiomer, which only weakly bound to β_1-adrenergic receptors, is completely different. For this compound, binding to S_{1A} sites accounts for 33%, β_1-adrenergic receptor binding for 22%, and β_2-adrenergic receptor binding, the veratridine site of the Na^+ channel, and the uptake of serotonine and dopamine between 7 and 10%.

The profile of various β-adrenergic blockers is shown in Tables 7 and 8. It reveals that carvedilol, pindolol, and propranolol also potently bind to S_{1A} binding sites with K_i values of 3, 15, and 84 nM, respectively. In addition, carvedilol and labetolol inhibited [³H]WB 401 binding to α_1-adrenergic receptor sites with a K_i value of 3 and 42 nM, respectively.

Discussion

Specificity of the β_1- and β_2-adrenergic receptor binding model. In agreement with previous reports (see Ref. 12)

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