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The Interaction of the Enantiomers of Carvedilol With α_1- and β_1-Adrenoceptors

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ABSTRACT The stereoselectivity of carvedilol, a novel β-adrenoceptor antagonist and vasodilator with one asymmetric carbon atom, was examined at α_1- and β_1-adrenoceptors in vitro and in vivo. (−)-(S)-Carvedilol is a potent, competitive antagonist of the β_1-adrenoceptor-mediated positive chronotropic response to isoproterenol in guinea pig atrium, with a dissociation constant (K_B) of 0.4 n*M*. (+)-(R)-Carvedilol was more than 100-fold less potent than the (−)-S-enantiomer as an antagonist of β_1-andrenoceptors, having a K_B of approximately 45 n*M*. Consistent with these findings (−)-(S)-carvedilol (0.1 mg/kg, i.v.) produced a 25-fold rightward shift in the β_1-adrenoceptor-mediated positive chronotropic response to isoproterenol in pithed rats, whereas the (+)-R-enantiomer had no β_1-adrenoceptor blocking activity in vivo at this dose. In contrast to the marked degree of stereoselectivity observed at β_1-adrenoceptors, both (−)-(S)- and (+)-(R)-carvedilol produced equal antagonism of the α_1-adrenoceptor-mediated vasoconstrictor response to norepinephrine in rabbit aorta, with K_B values of 14 and 16 n*M*, respectively. Furthermore, in the pithed rat, the α_1-adrenoceptor-mediated pressor dose–response curve to cirazoline was shifted approximately 6-fold to the right by both the (+)-R- and (−)-S-enantiomers of carvedilol at a dose of 1 mg/kg, i.v. In anesthetized spontaneously hypertensive rats, (−)-(S)-carvedilol was 6-fold more potent as an antihypertensive than (+)-(R)-carvedilol. The vasodilator and acute antihypertensive activity of carvedilol results from α_1-andrenoceptor blockade produced by both enantiomers, and the concomitant β_1-adrenoceptor blockade produced by the (−)-S-enantiomer, which prevents reflex tachycardia that can offset the antihypertensive response, leading to greater overall antihypertensive potency of (−)-(S)-carvedilol relative to the (+)-R-enantiomer. These data also suggest that distinct regions of the carvedilol molecule are responsible for blocking α_1- and β_1-adrenoceptors, with β_1-adrenoceptor blockade resulting from an area of the molecule containing the asymmetric carbon atom, specifically the carbazolyloxy propanolamine moiety, and α_1-adrenoceptor blockade resulting from a part of the molecule that does not contain the asymmetric carbon atom, most likely the phenoxyethylamine moiety.

INTRODUCTION

Carvedilol is a novel β-adrenoceptor antagonist and vasodilator that has antihypertensive activity in both animals[1] and humans.[2,3] Previous in vitro and in vivo studies have shown that carvedilol is a potent, relatively nonselective β_1- and β_2-adrenoceptor antagonist,[1,4,5] and an α_1-adrenoceptor antagonist.[4–6] Carvedilol is also a moderately potent calcium channel antagonist.[4,5,7] The antihypertensive activity of carvedilol most likely results from a combination of these activities, most important of which are the β- and α_1-adrenoceptor antagonist properties, since the antihypertensive activity in spontaneously hypertensive rats is nearly abolished by prior α_1- and β-adrenoceptor blockade with prazosin and propranolol, respectively.[4]

Carvedilol possesses one asymmetric carbon which gives rise to two enantiomers, and the preparation of carvedilol used clinically represents a 50:50 racemic mixture of the (−)-S- and (+)-R-enantiomers. Figure 1 shows the chemical structure of carvedilol and the position of the asymmetric carbon. Many agents that interact with α- and/or β-adrenoceptors possess asymmetric carbon atom(s), and the resulting enantiomers often show high degrees of stereoselectivity with respect to their interactions with the adrenoceptors, irrespective of

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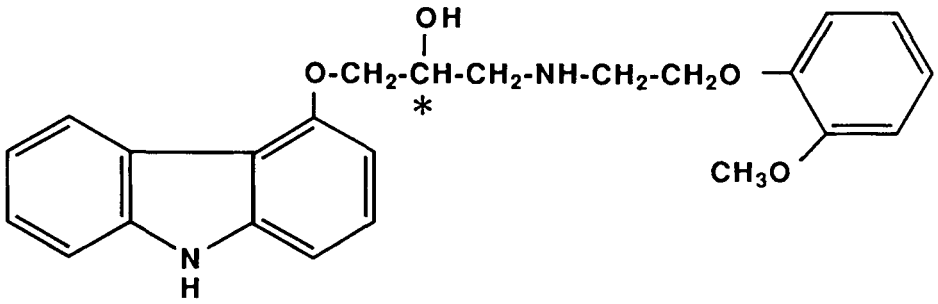

Carvedilol

Fig. 1. The chemical structure of carvedilol showing the position of the asymmetric carbon atom (*).

whether they are agonists or antagonists.[8,9] The purpose of the present study is to investigate the interaction of the enantiomers of carvedilol with α_1- and β_1-adrenoceptors in vitro and in vivo. In this way it may be possible to determine the stereochemical demands made by these receptors for carvedilol as well as to establish which parts of the molecule contribute to the α_1- and β_1-adrenoceptor antagonist properties of the drug.

MATERIAL AND METHODS

Isolated Tissues

Guinea pigs and rabbits were sacrificed by an overdose of sodium pentobarbital. Guinea pig atria and rabbit aorta were removed, dissected free of fat and connective tissue in Krebs–Henseleit buffer (see below for composition) and suspended in organ baths containing buffer aerated with 5% carbon dioxide–95% oxygen. The tissues were attached to Grass FT03 isometric transducers connected to either a Beckman R611 Dynograph, a Narco Bio-Systems Physiograph MK-IV, or a Narco Bio-Systems Narcotrace 80 recorder and allowed to equilibrate under a resting tension of either 1 g (guinea pig atrium) or 2 g (rabbit aorta) for at least 2 h before drug addition. The composition of Krebs–Henseleit buffer was (m*M*) NaCl, 118; KCl, 4.7; $MgCl_2$, 0.54; $CaCl_2$, 2.5; NaH_2PO_4, 1.0; $NaHCO_3$, 25; and glucose 11. Cocaine (6 μM) and EDTA (30 μM) were added to the Krebs–Henseleit buffer to block neuronal uptake and to prevent the spontaneous oxidation of catecholamines, respectively. The ability of the enantiomers of carvedilol to interact with α_1-adrenoceptors was assessed in the rabbit aorta using norepinephrine as the agonist,[10] and their ability to interact with β_1-adrenoceptors was assessed in the guinea pig atrium using isoproterenol as the agonist. Dose–response curves in all tissues were constructed by the method of stepwise cumulative addition of the agonist.[11] The concentration of the agonist in the organ bath was increased approximately 3-fold at each step, with each successive addition being made only after the response to the previous dose had attained a steady-state level and remained constant. After completion of a dose–response curve, drugs were washed from the preparation at regular intervals by the overflow method. Consecutive dose–response curves on a given tissue were separated by at least 2 h to ensure maximum washout of the agonist and to minimize the possibility of receptor desensitization. In all experiments at least one representative tissue was run in parallel with the experimental tissues but received neither of the enantiomers of carvedilol, and was used to correct for time-dependent changes in agonist sensitivity.[12]

Determination of Dissociation Constants

The dissociation constants (K_B) for the enantiomers of carvedilol acting as antagonists at α_1- and β_1-adrenoceptors were determined by the technique of Arunlakshana and Schild.[13] Dose ratios produced by the enantiomers of carvedilol (i.e., the ratio of the concentration of the agonist required to produce a half-maximal response in the presence of the antagonist divided by the control half-maximal concentration) were determined for various concentrations of the enantiomers of carvedilol. The logarithm of the (dose ratio − 1) was plotted against the respective logarithm of the concentration of carvedilol, which resulted in a straight line, allowing the slope and intercept along the abscissa to be determined. Under equilibrium conditions, the slope of the Schild regression does not differ significantly from unity if antagonism is competitive, and the intercept along the abscissa is the pA_2, which is equal to the negative logarithm of the dissociation constant (i.e., $-\log K_B$). When the slope of the Schild regression was not significantly different from unity, the data were fitted to a line with a slope constrained to 1.0, and the value of the intercept along the abscissa of this plot was used to determine the pA_2.[14,15]

Pithed Normotensive Rats

Male normotensive Sprague–Dawley rats (300–400 g, Charles River, Wilmington, DE) were anesthetized with sodium methohexital (60 mg/kg, i.p.). The tracheas were cannulated and the rats were pithed by passing a steel rod (1.5 mm in diameter) through the orbit and foramen magnum down into the spinal canal. Immediately after

pithing, the tracheal cannula was connected to a Harvard Apparatus model 680 rodent respirator and the rats were artificially ventilated at a frequency of 60 cycles/min with a tidal volume of 2 ml/100 g body weight. Systemic arterial blood pressure was measured from the right common carotid artery via a Statham P23 ID pressure transducer and was recorded on a Beckman R611 Dynograph recorder. Heart rate was measured by a Beckman 9857B cardiotachometer triggered by the pressure pulse and recorded on the Dynograph recorder. The left femoral vein was cannulated for the intravenous administration of drugs in a volume of 1 ml/kg. The preparation was allowed to stabilize for at least 30 min before drug administration. Under these conditions, diastolic blood pressure was 39 ± 1 mm Hg and heart rate was 304 ± 2 beats/min (n = 27).

To assess β_1-adrenoceptor antagonist activity, a cumulative dose–response curve was constructed for the positive chronotropic response to isoproterenol 15 min after pretreatment of the animals with either vehicle or one of the enantiomers of carvedilol. In the rat atrium isoproterenol-induced positive chronotropic responses are mediated exclusively by β_1-adrenoceptors.[16] To assess α_1-adrenoceptor antagonist activity, a cumulative dose–response curve was constructed for the pressor response to cirazoline 15 min after the intravenous administration of either vehicle or one of the enantiomers of carvedilol. Cirazoline-induced pressor responses in the pithed rat are mediated exclusively by α_1-adrenoceptors.[17]

Anesthetized Spontaneously Hypertensive Rats

Male, Okamoto-Aoki spontaneously hypertensive rats (SHR; 250–300 g, Taconic Farms, Germantown, NY) were anesthetized with sodium thiobutabarbital (120 mg/kg, i.p.) and the tracheas were cannulated to allow the rats to breathe room air spontaneously. The right carotid artery was cannulated for the measurement of systemic arterial blood pressure via a Statham P23 ID pressure transducer connected to a Beckman R611 Dynograph recorder. Heart rate was measured by a Beckman 9857B cardiotachometer triggered by the pressure pulse. The left jugular vein was cannulated for the administration of drugs in a volume of 1 ml/kg. The animals were allowed to stabilize for at least 30 min before drug administration. Under these conditions, resting mean arterial blood pressure was 146 ± 14 mm Hg (n = 8) and heart rate was 375 ± 15 beats/min (n = 8). Dose–response curves for the antihypertensive effects of the enantiomers of carvedilol were constructed by the method of stepwise cumulative administration of the compound. The cumulative dose of carvedilol was increased approximately 3-fold at each step, with each successive dose being given only when the response to the previous dose had attained a steady-state level.

Drugs

For in vitro studies, drugs were prepared as stock solutions (10 mM) in appropriate solvents, and subsequent dilutions were made in distilled water. The enantiomers of carvedilol (synthesized by Boerhinger-Mannheim GmbH, Mannheim, West Germany) were solubilized by dissolving the free base in N,N-dimethylformamide (DMF; final concentration, 10%), adding 1 mol equivalent of 1 N HCl and making up to volume in distilled water. (−)-Norepinephrine bitartrate and (−)-isoproterenol · HCl (Sigma Chemical Co., St. Louis, MO) were dissolved in distilled water containing ascorbic acid (50 mM). Cocaine · HCl (Merck, Sharpe & Dohme, West Point, PA) was dissolved in distilled water. For in vivo studies, stock solutions of the carvedilol enantiomers (3 mg/ml) were obtained by dissolving in DMF (final concentration, 10%), adding 1 mol equivalent of 1 N HCl and making up to volume in 0.9% NaCl. Sodium methohexital (Brevital; Eli Lilly and Co., Indianapolis, IN), sodium thiobutabarbital (Inactin; BYK Gulden, Konstanz, W. Germany), and cirazoline · HCl (gift from Synthelabo, Paris, France) were dissolved in 0.9% NaCl. Subsequent dilutions were made in 0.9% NaCl. The optical purity of the carvedilol enantiomers as determined by an HPLC assay system was (−)-(S)-carvedilol > 99.5%; (+)-(R)-carvedilol 98.8%.

Statistical Evaluation

All data are presented as the mean ± SEM of n observations. The statistical significance of the differences between means was tested by Student's t test for nonpaired observations.[18] All straight lines were drawn by linear regression[19] and tested, wherever possible, for deviations from linearity by analysis of variance in regression.[18] The slopes of the regression lines were tested for significance by an F test.[19]

RESULTS

Isolated Tissues

(−)-(S)-Carvedilol produced a potent, competitive inhibition of the β_1-adrenoceptor mediated positive chronotropic response to isoproterenol in the guinea atrium (Fig. 2A). Analysis of the data by the method of Arunlakshana and Schild[13] gave a Schild regression with slope of 1.23 ± 0.16, which was not significantly different from the theoretical value of unity. The Schild regression with slope constrained to unity gave a pA_2 of 9.44 ± 0.07, which corresponds to a K_B of 0.4 nM. In contrast, (+)-(R)-carvedilol produced only a very small inhibition of the response to isoproterenol at 0.1 μM (Fig. 2B). At this concentration, (+)-(R)-carvedilol produced a dose ratio of 3.9 ± 0.9, which corresponds to a K_B of 45 nM.

In contrast to the marked stereoselectivity observed at β_1-adrenoceptors, Figure 3 shows that both enantiomers of carvedilol were potent, competitive antagonists of the α_1-adrenoceptor-mediated vasoconstrictor response to norepinephrine in rabbit aorta. (−)-(S)-Carvedilol gave a Schild regression with a slope of 0.86 ± 0.14, which was not significantly different from unity. The Schild regression with slope constrained to 1.0 gave a pA_2 of 7.87 ± 0.06, which corresponds to a

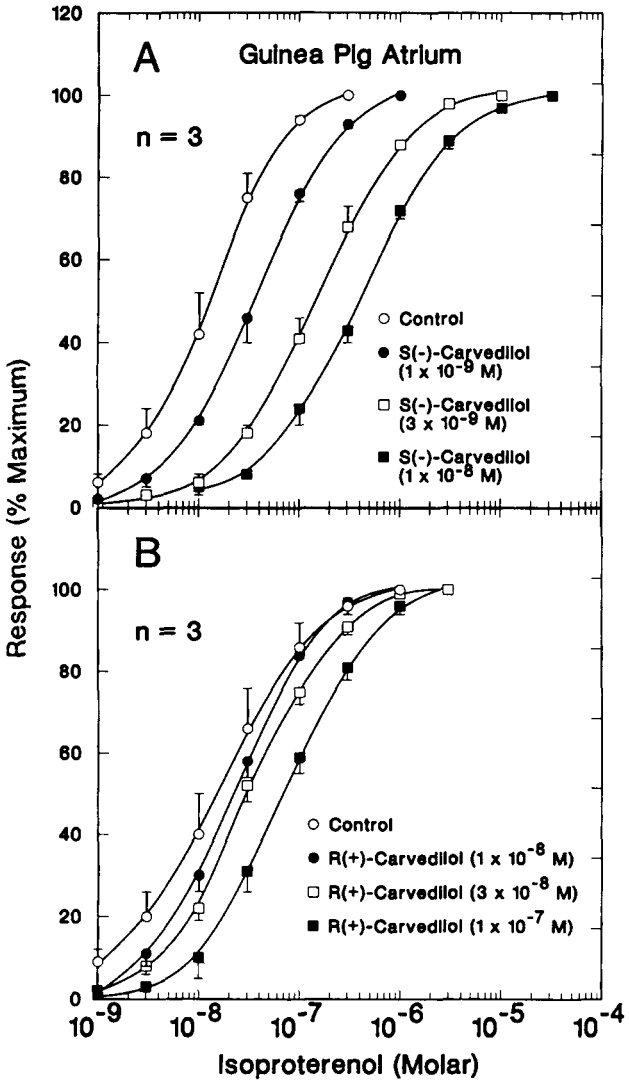

Fig. 2. The β_1-adrenoceptor antagonist activity of (−)-(S)-carvedilol (**A**) and (+)-(R)-carvedilol (**B**) in guinea pig isolated spontaneously beating atrium.

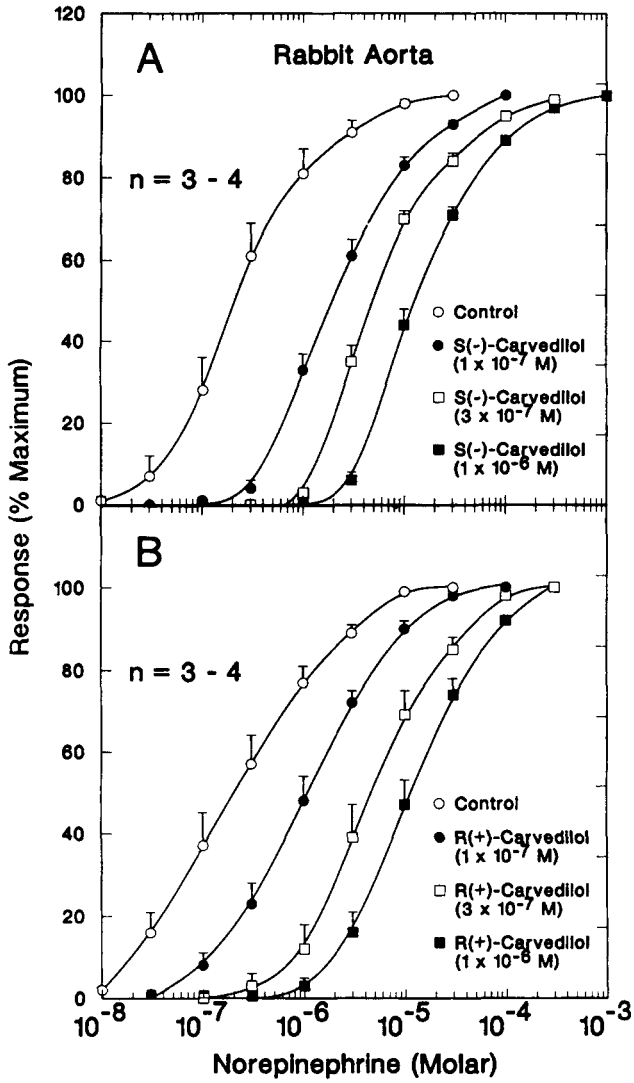

Fig. 3. The α_1-adrenoceptor antagonist activity of (−)-(S)-carvedilol (**A**) and (+)-(R)-carvedilol (**B**) in rabbit isolated aorta.

K_B of 13 nM. Similarly, the Schild regression for (+)-(R)-carvedilol had a slope (1.10 ± 0.25) that was not significantly different from unity. The Schild regression with slope constrained to 1.0 gave a pA_2 of 7.79 ± 0.10, which corresponds to a K_B of 16 nM. The dissociation constants for the two enantiomers of carvedilol at α_1-adrenoceptors in rabbit aorta were not significantly different from each other ($p > 0.05$).

Pithed Rats

In pithed rats, the dose–response curve for the β_1-adrenoceptor-mediated positive chronotropic response to isoproterenol was shifted 25-fold to the right by (−)-(S)-carvedilol at a dose of 0.1 mg/kg, i.v. (Fig. 4). In contrast, (+)-(R)-carvedilol at the same dose did not produce significant β_1-adrenoceptor blockade (Fig. 4), confirming the stereoselectivity observed for the enantiomers of carvedilol in vitro.

Both enantiomers of carvedilol, at a dose of 1 mg/kg, i.v., produced a 6-fold rightward shift of the dose–response curve for the α_1-adrenoceptor mediated pressor response to cirazoline (Fig. 5), confirming the lack of stereoselectivity observed for the enantiomers of carvedilol in vitro.

Anesthetized Spontaneously Hypertensive Rats

The antihypertensive activity of the enantiomers of carvedilol was studied in anesthetized spontaneously hypertensive rats. As may be seen in Figure 6, both enantiomers of carvedilol are potent antihypertensive agents

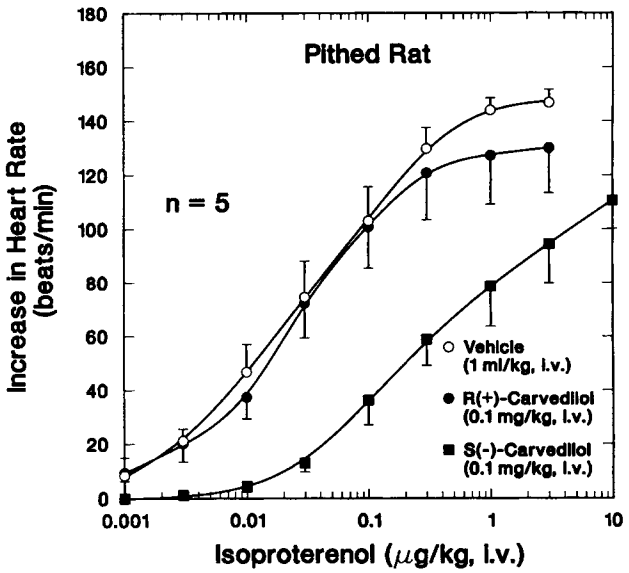

Fig. 4. The β_1-adrenoceptor antagonist activity of (−)-(S)- and (+)-(R)-carvedilol (1 mg/kg, i.v., −15 min) in pithed rats.

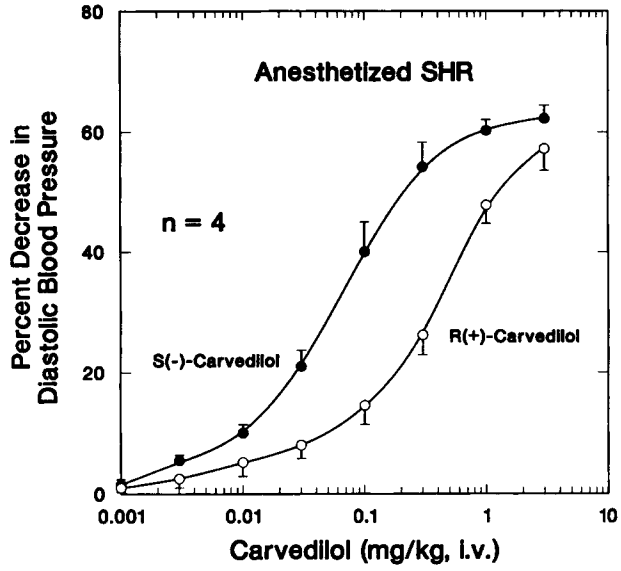

Fig. 6. The hypotensive activity of (−)-(S)- and (+)-(R)-carvedilol in anesthetized spontaneously hypertensive rats.

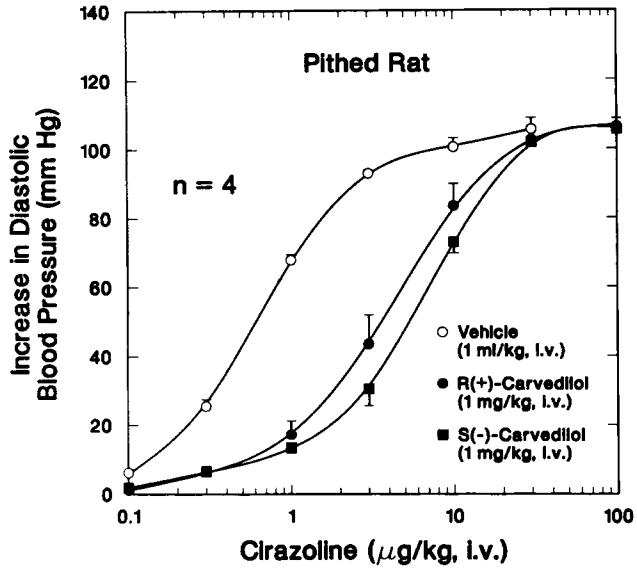

Fig. 5. The α_1-adrenoceptor antagonist activity of (−)-(S)- and (+)-(R)-carvedilol (1 mg/kg, i.v., -15 min) in pithed rats.

in vivo, with (−)-(S)-carvedilol being approximately 6-fold more potent than (+)-(R)-carvedilol.

DISCUSSION

These data clearly demonstrate, both in vitro and in vivo, that the enantiomers of carvedilol show marked stereoselectivity at β_1-adrenoceptors, but no stereochemical difference at α_1-adrenoceptors. As such, the two enantiomers of carvedilol have markedly different pharmacologic profiles. The (−)-S-enantiomer of carvedilol is a potent β_1-adrenoceptor antagonist and an α_1-adrenoceptor antagonist, whereas the (+)-R-enantiomer is a selective α_1-adrenoceptor antagonist. The stereoselectivity observed at the β_1-adrenoceptor was in accord with previous studies of other β-adrenoceptor antagonists,[8] with the (−)-(S)-enantiomer being significantly more potent than the (+)-R-enantiomer. The dissociation constant of (−)-(S)-carvedilol for β_1-adrenoceptors in guinea pig atrium (0.4 nM) is one-half of the value reported by us previously for the racemic mixture (0.8 nM [5]), which is consistent with what would be expected for a racemic mixture in which one of the two enantiomers was essentially inactive. The weak β_1-adrenoceptor blocking activity observed at high concentrations of (+)-(R)-carvedilol may be due to the small amount (approximately 1%) of the (−)-S-enantiomer that was present in the sample after resolution, and not to a direct action of the (+)-R-enantiomer itself. The dissociation constants obtained for the two enantiomers acting at α_1-adrenoceptors [(−)-(S) 14 nM; (+)-(R) 16 nM] were not significantly different from those obtained by us previously for the racemic mixture (10 nM [5]).

The fact that the enantiomers of carvedilol show differential stereoselectivity at α_1- and β_1-adrenoceptors strongly suggests that different parts of the carvedilol molecule are responsible for the α_1- and β_1-adrenoceptor blocking activities. Examination of the structure of carvedilol (see Fig. 1) shows the molecule to be an aryloxypropanolamine, specifically carbazol-4-yloxy-2-propanolamine, attached to a phenoxyalkylamine, 2-methoxyphenoxyethylamine, with the amine group shared by both parts. The asymmetric carbon atom of

carvedilol is within the aryloxypropanolamine moiety. Aryloxypropanolamines form a general class of β-adrenoceptor antagonists, such as propranolol. Thus, the fact that the carbazolyoxypropanolamine moiety is similar to that found in other β-adrenoceptor antagonists, and that stereoselectivity is seen for the β_1-adrenoceptor blocking activity of carvedilol which possesses an asymmetric carbon atom in the carbazolyloxypropanolamine portion of the molecule, strongly suggests that this part of the molecule is responsible for the β_1- (and presumably the β_2-) adrenoceptor antagonist activity of carvedilol.

Because there is no stereoselectivity for the α_1-adrenoceptor antagonist property of carvedilol, it would appear that this activity does not result from the carbazolyloxypropranolamine moiety, but rather results from the phenoxyethylamine moiety. Phenoxyalkylamines form a general class of α-adrenoceptor antagonists,[20] and many phenoxyethylamines are potent, selective α_1-adrenoceptor antagonists, such as thymoxamine, phenoxybenzamine, and WB-4101. Phenoxybenzamine and WB-4101 have chiral centers in the phenoxyethylamine moiety, and both compounds show stereoselectivity with respect to their ability to interact with α_1-adrenoceptors.[9] This is in contrast to the lack of stereoselectivity of the carvedilol enantiomers at α_1-adrenoceptors, which we interpret as being due to the absence of an asymmetric carbon atom in the phenoxyethylamine moiety of carvedilol.

The functional significance of the difference in the α_1- and β_1-adrenoceptor blocking activities of the enantiomers of carvedilol is demonstrated in the difference observed in the antihypertensive potency. Both enantiomers of carvedilol, which block α_1-adrenoceptors equally well, lower blood pressure, with the (−)-S-enantiomer, which also blocks β_1-adrenoceptors, being approximately 6-fold more potent than the (+)-R-enantiomer. This finding adds support to the notion that β_1-adrenoceptor blockade can augment the antihypertensive response to a vasodilator. In other words, for any given degree of vasodilation, concomitant β-adrenoceptor blockade will produce a greater reduction in blood pressure due to blockade of reflex tachycardia. In addition, β-adrenoceptor blockade may also enhance the hypotensive response to α_1-adrenoceptor blockade by other mechanisms, i.e., inhibition of renin release or a central mechanism. Also, the degree of vasodilator activity required to produce a given reduction in blood pressure will be less in the presence of concomitant β_1-adrenoceptor blockade.

Our data indicate that the enantiomers of carvedilol show differential stereoselectivity as antagonists at α_1- and β_1-adrenoceptors, with no stereoselectivity observed at α_1-adrenoceptors, but marked stereoselectivity observed at β_1-adrenoceptors, with the (−)-S-enantiomer being over 100-fold more potent than the (+)-R-enantiomer. A functional consequence of these observations is that the (−)-S- and (+)-R-enantiomers of carvedilol have dramatically different pharmacologic profiles, and that the pharmacologic profile of the racemic mixture of carvedilol used clinically cannot be obtained with either of the enantiomers alone.

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