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European Journal of Pharmacology 481 (2003) 83–89
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Carvedilol blockade of rat myocardial α_1-adrenoceptors

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Received 12 June 2003; received in revised form 27 August 2003; accepted 3 September 2003

Abstract

Carvedilol is a combined α_1- and β-adrenoceptor antagonist. The ability of carvedilol to antagonize functional effects mediated through myocardial α_1-adrenoceptors has never been investigated. We tested the ability of carvedilol to antagonize the inotropic effect mediated by myocardial α_1-adrenoceptors compared to the antagonism of β-adrenoceptors. Papillary muscles from rat heart left ventricle were mounted in an organ bath and concentration–response experiments for the inotropic effects of separate α_1- and β-adrenoceptor stimulation were performed in the absence and presence of carvedilol. Carvedilol antagonized myocardial α_1-adrenoceptors with an inhibition constant (K_i) of 11.0 ± 3.0 nmol/l and the functional experiments were supported by radioligand-binding studies. Corresponding functional studies on the response to β-adrenoceptor stimulation revealed a K_i of 1.2 ± 0.35 nmol/l. Thus, carvedilol antagonizes the myocardial α_1-adrenoceptors with a 9-fold lower potency than the β-adrenoceptors. Antagonism of myocardial α_1-adrenoceptor evoked effects may contribute to clinical effects of carvedilol.

Keywords: Carvedilol; α_1-Adrenoceptor; β-Adrenoceptor; Myocardium, rat

1. Introduction

Clinical trials have shown that carvedilol offers a highly rational approach in the management of congestive heart failure, hypertension and myocardial infarction (Dargie, 2001; Dunn et al., 1997; Eichhorn and Bristow, 2001).

Carvedilol is essentially a nonselective β-adrenoceptor antagonist which lacks intrinsic sympathomimetic activity (Brixius et al., 2001) and has anti-oxidative and anti-proliferative effects (Ohlstein et al., 1993; Yue et al., 1992). In addition, carvedilol has vasodilatory effects attributable to its α_1-adrenoceptor blocking properties (Sponer et al., 1992). Accordingly, carvedilol distinguishes itself from other β-adrenoceptor antagonists providing a more comprehensive adrenoceptor blockade. It is unclear whether this pharmacological property of carvedilol can translate into superior clinical effects compared to other β-adrenoceptor antagonists but studies may suggest that carvedilol confers additional benefits over conventional β-adrenoceptor blockade (Metra et al., 2000; Packer et al., 2001).

The β-adrenoceptor antagonizing effect of carvedilol has been thoroughly investigated in the mammalian myocardium (Brixius et al., 2001; Maack et al., 2000; Yoshikawa et al., 1996). Despite the fact that α_1-adrenoceptors are present in the myocardium, the ability of carvedilol to antagonize α_1-adrenoceptors is mainly investigated and emphasized with regard to its peripheral effects on vascular musculature (Monopoli et al., 1989). To our knowledge, the ability of carvedilol to antagonize functional effects mediated through myocardial α_1-adrenoceptors has never been investigated although α_1-adrenoceptors have been identified in the myocardium in most mammalian species and play a role in the control of cardiac function under physiological and pathophysiological conditions (Terzic et al., 1993). Activation of myocardial α_1-adrenoceptors mediates a positive inotropic response, as well as alterations in ion movements and metabolic responses of the myocardium (Andersen et al., 1998; Skomedal et al., 1997; Terzic et al., 1993). These effects indicate that myocardial α_1-adrenoceptors play functional roles in normal myocardium. In pathological condi-

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doi:10.1016/j.ejphar.2003.09.001

tions as heart failure, myocardial α_1-adrenoceptors may represent a compensatory mechanism to maintain cardiac inotropic support when the β-adrenoceptor mediated responses are attenuated (Sjaastad et al., 2003; Skomedal et al., 1997). Myocardial α_1-adrenoceptors might also mediate ischemic preconditioning (Salvi, 2001). In addition, α_1-adrenoceptors can promote hypertrophic signalling and chronic α_1-adrenoceptor stimulation induces a hypertrophic phenotype in cultured neonatal and adult rat cardiomyocytes (Ponicke et al., 2001; Simpson, 1983). Cardiac overexpression of α_{1B}-adrenoceptors in transgenic mice displays a phenotype consistent with cardiac hypertrophy (Milano et al., 1994). In addition, cathecholamines seem to be required for the development of cardiac hypertrophy in vivo (Rapacciuolo et al., 2001). Accordingly, activation of myocardial α_1-adrenoceptors can provide both favourable and deleterious effects to the heart raising uncertainty about the importance of antagonism of myocardial α_1-adrenoceptors.

The aim of the present study was to investigate the ability of carvedilol to antagonize the inotropic response induced by myocardial α_1-adrenoceptors and compare such an antagonism to the effect of carvedilol on β-adrenoceptors. In addition, receptor radioligand binding of α_1-adrenoceptors was performed for validation of the results from the functional studies. Accordingly, this will elucidate the effect of carvedilol on the balance between the α_1- and the β-adrenergic receptor system in the myocardium.

2. Materials and methods

2.1. Animals

Animals were cared for according to the Norwegian Animal Welfare Act which conforms with the European Convention for the protection of Vertebrate animals used for Experimental and other Scientific Purposes (Council of Europe no. 123, Strasbourg 1985). Two animals were kept in each cage and housed in a temperature-regulated room with a 12-h day/12-h night cycling and allowed free access to water and standard rat chow.

2.2. Isolated papillary muscles

Male, Wistar rats (Møllegaard Breeding and Research Centre, Skensved, Denmark), weighing about 320 g were anaesthetized and ventilated on a respirator with 2.2% Isoflurane and the heart was carefully isolated, dissected free of connective tissue and transferred to ice-cold 0.9% NaCl.

The aorta was cannulated and the coronaries were perfused at 31 °C (pH 7.4) with a salt solution described below. Posterior left ventricular papillary muscle was ligated and dissected free and mounted in organ baths. In order to prevent contracture of the papillary muscles during dissection and mounting, we used a relaxing solution with a Ca^{2+}/Mg^{2+} concentration ratio of 1:8 comparable to that of St. Thomas' Hospital cardioplegic solution. The relaxing solution contained (mmol/l): NaCl 118.3; KCl 3.0; $CaCl_2$ 0.5; $MgSO_4$ 4.0; KH_2PO_4 2.4; $NaHCO_3$ 24.9; glucose 10.0; mannitol 2.2 and equilibrated with 95% O_2/5% CO_2 at 31 °C (pH 7.4).

The papillary muscles were mounted in organ baths containing the relaxing solution and allowed to adapt at 31 °C for about 20 min before the solution was changed to one containing the following (in mmol/l): NaCl 119.2; KCl 3.0; $CaCl_2$ 2.0; $MgSO_4$ 1.2; KH_2PO_4 2.4; $NaHCO_3$ 24.9; glucose 10.0; mannitol 2.2 and equilibrated with 95% O_2/5% CO_2 at 31 °C (pH 7.4).

The muscles were field stimulated with alternating polarity at 1 Hz with impulses of 5 ms duration and current about 20% above individual threshold (10–15 mA, determined in each experiment). The isometrically contracting muscles were stretched to the maximum of their length–tension curve. The force was recorded and analysed as previously described (Skomedal et al., 1997). The muscles were allowed to equilibrate for 90 min. When used, prazosin, timolol and carvedilol were allowed to act for 90 min before addition of agonist.

Signal averaged contraction–relaxation cycles were calculated for different experimental periods, and these cycles were used to determine the inotropic response expressed as increase in maximal development of force (dF/dt_{max}). Contraction–relaxation cycles at the end of the equilibrium period were used as control.

2.3. Experimental design

Pure α_1- and β-adrenoceptor mediated inotropic responses were obtained in the presence of appropriate receptor antagonists. Accordingly, either the β-adrenoceptor antagonist timolol (1 μmol/l) or the α_1-adrenoceptor antagonist prazosin (0.1 μmol/l) was used in all experiments. At these concentrations, the receptor antagonists prevented the response to different agonists through their respective receptor system.

The concentration–response experiments for α_1-adrenoceptor stimulation by phenylephrine and noradrenaline were performed in the absence and presence of 20 or 80 nmol/l of carvedilol, respectively.

The concentration–response experiments for β-adrenoceptor stimulation by isoprenaline were performed in the absence and presence of 5 or 20 nmol/l carvedilol, respectively. The presence of antagonists did not influence the basal contraction–relaxation cycles characteristics or electrical stimulation threshold (data not shown).

The different agonists were added directly to the organ baths in increasing concentrations until supramaximal concentration of agonist was obtained with respect to inotropic response. The time to maximal inotropic response after adding agonist to the baths was 5–10 min during α_1-adrenoceptor stimulation and 3–5 min during β-adrenoceptor stimulation.

2.4. Receptor binding

The heart tissue was immediately snapfrozen in liquid nitrogen and stored at −80 °C. When used, the tissue was pulverized in a stainless steel mortar precooled in liquid nitrogen. Pulverized frozen heart tissue was homogenized with 50 mmol/l Tris–HCl, 10 mmol/l $MgCl_2$, pH 7.4. An equal volume of 1 mol/l KCl was added to the homogenate, which was left on ice for 10 min followed by centrifugation at 51.500 × *g* for 12 min. The pellet was resuspended in homogenization buffer with an Ultra-Turrax, and the homogenate was recentrifuged. This washing procedure was repeated twice. α_1-Adrenoceptor binding of carvedilol was determined using 0.3 nmol/l [7-methoxy- ^{3}H]prazosin (specific activity 80 Ci $mmol^{-1}$) as radioligand and increasing concentrations of carvedilol. Phentolamine (10 μmol/l) was used to determine nonspecific binding. The nonspecific binding of [^{3}H]prazosin was 10.8 ± 1.0% of total binding (n = 13) and the equilibrium dissociation constant of the radioligand was 156 ± 25 pmol/l. The binding reaction was performed at 37 °C for 30 min. The reaction was stopped by addition of 1.5 ml 0.1% γ-globulin in 0.1 mol/l NaCl and 1 ml 20% polyethyleneglycol and further processed as described by Iyengar et al. (1980).

2.5. Calculation and statistics

The inotropic responses to either agonist are presented as percentage of initial maximal development of force (dF/dt). When appropriate the values after responses to agonist were also expressed as percent of maximal response (100%). The concentration–response curves from papillary muscle experiment were constructed according to Ariëns et al. (1964), by estimating centiles (EC_{10} to EC_{100}) for each single experiment and calculating the corresponding means. This calculation provides mean curves that express the response as fractional response or percent of maximum and display horizontal positioning and the correct mean slope of the curves. The horizontal positions of the concentration–response curves were expressed as pD_2-values ($-\log EC_{50}$).

Carvedilol displacement of [^{3}H]prazosin was analyzed by using GraphPad Prism® version 4.03 (GraphPad Software, CA, USA) and the centile technique was applied for calculating the curve presented (Fig. 4).

Data are expressed as mean ± standard error of the mean (S.E.M.) and the number of animals expressed as n. The significance levels of differences were calculated according to Student's t-test. $P < 0.05$ is considered to indicate a statistically significant difference. When appropriate, Bonferroni corrections were made in order to keep the level of significance. In the functional experiments the inhibition constant (K_i) for carvedilol was calculated from the Schild equation, based on the relative shift of the concentration–response curves for receptor stimulation. The shifts of the curves were expressed as ΔpD_2-values and the corresponding dose-ratios were used in the Schild equation. In the radioligand-binding experiments, the inhibition constant for carvedilol was calculated according to Cheng and Prusoff (1973).

2.6. Drugs

Carvedilol was kindly supplied by Roche Diagnostics (Mannheim, Germany). Prazosin hydrochloride, Timolol bitartrate, (−)-Phenylephrine hydrochloride and (−)-Isoprenaline hemisulphate were purchased from Sigma. (−)-Noradrenaline bitartrate was purchased through Norwegian Medical Depot. Stock solutions were prepared in double-distilled water and kept at −20 °C to avoid oxidation. Further solutions of the drugs were made fresh daily and kept cool (0–4 °C).

3. Results

3.1. α_1-Adrenoceptor blockade by carvedilol

Concentration–response curves for the pure α_1-adrenoceptor induced inotropic responses were obtained for phenylephrine or the endogenous agonist noradrenaline in the presence of 1 μmol/l timolol. The concentration–response curves were shifted to higher concentrations of agonist by 20 and 80 nmol/l carvedilol, respectively.

3.1.1. Phenylephrine as agonist

α_1-Adrenoceptor mediated maximal inotropic response induced by phenylephrine was 50.2 ± 3.3% (n = 26, pooled average), and there were no statistically significant differences between the maximal inotropic responses in the three groups exposed to phenylephrine (Table 1).

α_1-Adrenoceptor stimulation by phenylephrine in the absence of carvedilol exhibited a pD_2-value of 5.23 ± 0.08 (n = 8) (Table 1). In the presence of 20 (n = 11) nmol/l and 80 (n = 8) nmol/l carvedilol, the concentration–response curves were shifted in a parallel manner to higher concentrations of

Table 1
Characteristics of adrenoceptor mediated responses and antagonism of carvedilol

Agonist/+antagonist	Max effect ± S.E.M. (%)	pD_2 ± S.E.M.	K_i ± S.E.M. (nmol/l)
Phenylephrine (PE)	57.3 ± 4.7	5.23 ± 0.08	–
PE + 20 nmol/l Carv	52.7 ± 5.8	4.78 ± 0.07	11.0 ± 3.1
PE + 80 nmol/l Carv	40.5 ± 5.1	4.28 ± 0.07	10.0 ± 2.4
Noradrenaline (NA)	47.1 ± 7.5	5.42 ± 0.07	–
NA + 20 nmol/l Carv	53.4 ± 2.9	4.94 ± 0.05	10.0 ± 3.0
NA + 80 nmol/l Carv	53.7 ± 5.2	4.57 ± 0.07	12.5 ± 3.8
Isoprenaline (Iso)	139.1 ± 10.2	6.82 ± 0.07	–
Iso + 5 nmol/l Carv	113.2 ± 14.6	6.08 ± 0.06	1.10 ± 0.32
Iso + 20 nmol/l Carv	99.0 ± 4.2	5.59 ± 0.04	1.25 ± 0.37

Maximal inotropic effect above basal (Max effect) and pD_2 (= pEC_{50}) for α_1-adrenoceptor (Phenylephrine/Noradrenaline) and β-adrenoceptor (Isoprenaline) stimulation in the absence and presence of 5, 20 or 80 nmol/l carvedilol (Carv) and corresponding inhibition constants (K_i) for carvedilol.

agonist by 0.45 ± 0.11 ($P < 0.05$) and 0.95 ± 0.11 ($P < 0.05$) log units, respectively (Fig. 1, Table 1). The corresponding inhibition constants (K_i's) calculated from the Schild equation were 11.0 ± 3.1 and 10.0 ± 2.4 nmol/l, respectively. The calculated K_i-values corresponded to a slope near unity (1.06) in a Schild plot, thus verifying competitive antagonism for carvedilol on the inotropic response elicited by stimulation of myocardial α_1-adrenoceptors.

3.1.2. Noradrenaline as agonist

The maximal α_1-adrenoceptor mediated inotropic response to noradrenaline was 51.4 ± 3.0% ($n = 28$, pooled average), and there were no statistically significant differences between the maximal inotropic responses in the three groups exposed to noradrenaline (Table 1). Nor was there significant difference between the maximal α_1-adrenoceptor mediated inotropic responses elicited by noradrenaline and phenylephrine.

In the absence of carvedilol, the α_1-adrenoceptor stimulation by noradrenaline exhibited a pD_2-value of 5.42 ±0.07 ($n = 9$) (Table 1). The concentration–response curves were shifted by 0.48 ± 0.08 ($P < 0.05$) and 0.85 ± 0.09 ($P < 0.05$) log units by 20 nmol/l ($n = 10$) and 80 nmol/l ($n = 9$) carvedilol, respectively (Fig. 2, Table 1). The inhibition constants (K_i's) calculated from the Schild equation were 10.0 ± 3.0 and 12.5 ± 3.8 nmol/l, respectively.

3.2. β-Adrenoceptor blockade by carvedilol

Concentration–response curves for β-adrenoceptor-mediated inotropic response were obtained by adding cumulatively isoprenaline in the presence of 0.1 μmol/l prazosin. The concentration–response curves were shifted in a parallel manner to a higher concentration of isoprenaline by 5 and 20 nmol/l carvedilol, respectively.

The mean maximal inotropic response induced by isoprenaline was 115.5 ± 6.4% ($n = 23$, pooled average). Ap-

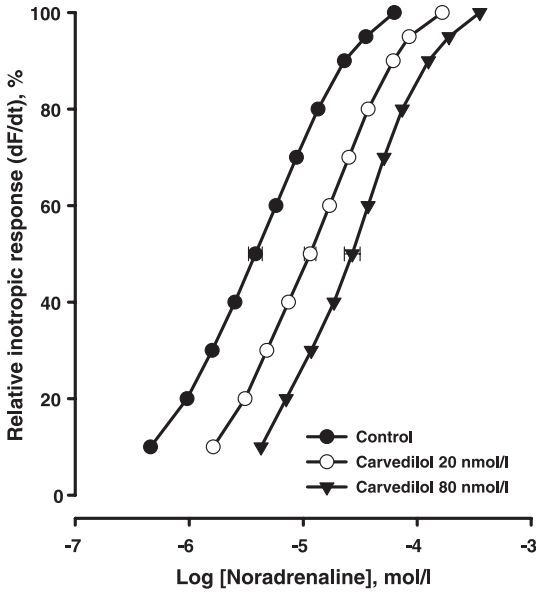

Fig. 2. The concentration–response curves for α_1-adrenoceptor stimulation by noradrenaline in the absence and presence of 20 or 80 nmol/l carvedilol, respectively. Ordinate; Inotropic response (increase in dF/dt) expressed in percent of maximum. Abscissa; logarithmic concentration of noradrenaline. Horizontal error bars represent S.E.M. of pEC_{50}.

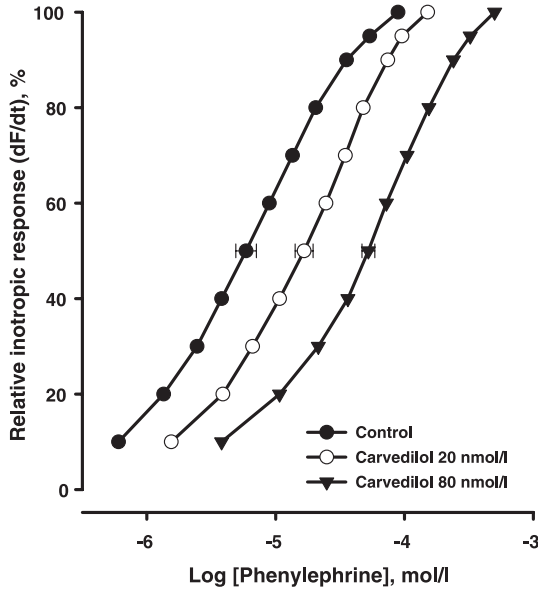

Fig. 1. The concentration–response curves for α_1-adrenoceptor stimulation by phenylephrine were essentially parallel in the absence and presence of 20 or 80 nmol/l carvedilol, respectively. Ordinate; Inotropic response (increase in dF/dt) expressed in percent of maximum. Abscissa; logarithmic concentration of phenylephrine. Horizontal error bars represent S.E.M. of pEC_{50}.

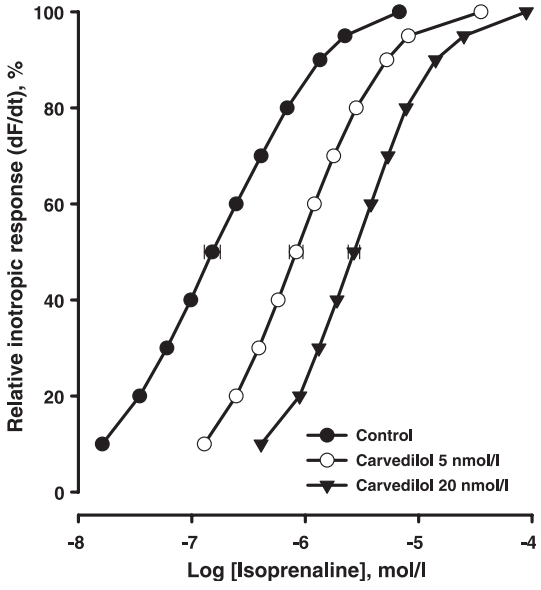

Fig. 3. The concentration–response curves for β-adrenoceptor stimulation by isoprenaline in the absence and presence of 5 or 20 nmol/l carvedilol, respectively. Ordinate; Inotropic response (increase in dF/dt) expressed in percent of maximum. Abscissa; logarithmic concentration of isoprenaline. Horizontal error bars represent S.E.M. of pEC_{50}.

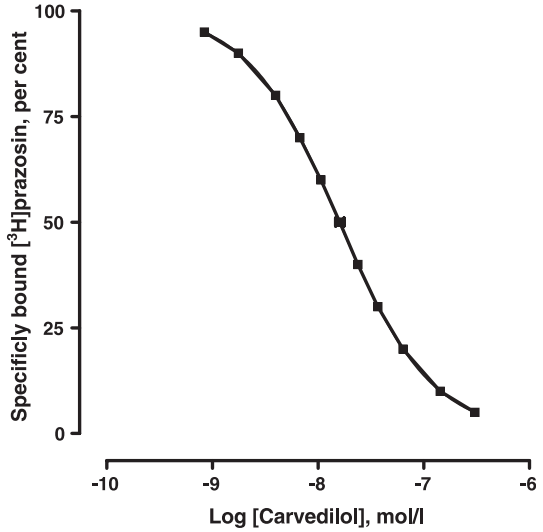

Fig 4. Inhibition of specific [^{3}H]prazosin binding by carvedilol. The curve represents an average of individual experiments ($n=13$) and is constructed by a centile technique. $pIC_{50}=7.8\pm0.04$. Ordinate; specifically bound [^{3}H]prazosin in percent of binding without carvedilol. Abscissa; logarithmic concentration of carvedilol. S.E.M. of pIC_{50} is hidden within the symbol.

parently, there was some variation in the response of the different groups exposed to isoprenaline although the same criteria for reaching maximal inotropic response were used (Table 1) (see Materials and methods).

β-Adrenoceptor stimulation by isoprenaline in the absence of carvedilol exhibited a pD_2-value of 6.82 ± 0.07 ($n=7$) (Table 1). In the presence of 5 nmol/l ($n=9$) and 20 nmol/l ($n=7$) carvedilol, the concentration–response curves were shifted 0.74 ± 0.06 ($P<0.05$) and 1.23 ± 0.08 ($P<0.05$) log units, respectively (Fig. 3, Table 1). The corresponding inhibition constants (K_i's) calculated from the Schild equation were 1.10 ± 0.32 and 1.25 ± 0.37 nmol/l, respectively.

3.3. α_1-Adrenoceptor binding of carvedilol

α_1-Adrenoceptor radioligand-binding studies were performed on a particulate fraction from the heart in the presence of 0.3 nmol/l [^{3}H]prazosin and appropriate concentrations of carvedilol. The binding curves obtained were best fitted with a one-site binding model (Fig. 4). Carvedilol was able to inhibit the specific binding of [^{3}H]prazosin to the α_1-adrenoceptors with a calculated K_i value of 5.65 ± 0.34 nmol/l ($n=13$).

4. Discussion

The novel finding in this study is that carvedilol exerts a functional antagonism of myocardial α_1-adrenoceptors with a K_i of 11.0 ± 3.0 nmol/l (pooled result phenylephrine and noradrenaline, $n=55$). A direct comparison in the same experimental system between the potency of carvedilol as an α_1-adrenoceptor antagonist and its properties as a β-adrenoceptor antagonist was performed. Accordingly, myocardial β-adrenoceptors were antagonized with a K_i of 1.2 ± 0.35 nmol/l (pooled result isoprenaline, $n=23$) suggesting that carvedilol antagonizes the myocardial α_1-adrenoceptors with a 9-fold lower potency than the β-adrenoceptors. Compared to the high-affinity competitive α_1-adrenoceptor antagonist prazosin (K_i value of 0.04 nmol/l) (Skomedal et al., 1980), carvedilol exhibits a moderate antagonism of myocardial α_1-adrenoceptors. The concentration–response curves were shifted to a higher concentration of agonist in a parallel manner and the maximal inotropic response to phenylephrine and noradrenaline did not differ in the absence or presence of carvedilol. The inhibition constants calculated for the two concentrations of carvedilol were similar irrespective of agonist used (Table 1). Accordingly, carvedilol acts as a competitive antagonist on the inotropic response elicited by stimulation of myocardial α_1-adrenoceptors and the inhibition constants calculated from functional studies were supported by that derived form radioligand-binding studies.

In the rat heart, three α_1-adrenoceptor subtypes have been detected at the mRNA level and at the protein level binding studies in the rat have revealed a α_{1A} to α_{1B} ratio of 20% to 80% (Michel et al., 1994, 1995). There is still uncertainty whether α_{1D} can be detected at the protein level (Deng et al., 1996; Yang et al., 1997).

Carvedilol displayed no ability to recognize an inhomogeneous receptor population in our experiments and the competition curves for carvedilol were best fitted by an one-site binding model. Accordingly, we suggest that carvedilol is nonselective with respect to α_1-adrenoceptor subtypes.

Carvedilol antagonizes vascular α_1-adrenoceptors in vitro and in vivo. Carvedilol produced a competitive inhibition of α_1-adrenoceptor mediated contractile response to noradrenaline in rabbit aorta and human internal mammary artery with a K_b of 11 and 2.5 nmol/l, respectively (Monopoli et al., 1989; Nichols et al., 1989a). In conscious spontaneously hypertensive rats, carvedilol produced reduction in blood pressure associated with a reduction in total peripheral vascular resistance attributable to its α_1-adrenoceptor blocking properties (Nichols et al., 1991) and in humans carvedilol demonstrated in vivo peripheral vasodilating actions (Metra et al., 1994).

To our knowledge, the present study is the first to demonstrate a functional antagonism of myocardial α_1-adrenoceptors by carvedilol and the functional data are supported by radioligand-binding studies. Thus, the functional inhibition constant for carvedilol on myocardial α_1-adrenoceptors is in good agreement with the results for vascular α_1-adrenoceptors receptors as well as radioligand-binding studies performed on human ventricular myocardi-

um (Bristow et al., 1992). This suggests that in vivo vasodilating effect of carvedilol is accompanied by a similar antagonism of myocardial α_1-adrenoceptor-mediated effects in clinical doses.

Carvedilol has an asymmetric carbon atom giving rise to equal amounts of two enantiomers. Both enantiomers exhibit similar α_1-adrenoceptor blocking properties while the *S*-(−)-enantiomer is preferentially a β-adrenoceptor antagonist ($K_b = 0.4$ nM), approximately 100-fold more potent than the *R*-(+)-enantiomer ($K_b = 45$ nM) (Nichols et al., 1989b). Pharmacokinetic studies show that the concentration of *R*-(+)-enantiomer exceeds the concentration of the *S*-(−)-enantiomer especially after per os administration (Spahn et al., 1990) suggesting a relative increase in the ratio between α_1- and the β-adrenoceptor blockade by a faster reduction of the latter.

Taken into account the protein binding of (*R*,*S*)-carvedilol, the concentrations of the non-bound form of the drug in clinical doses is estimated to be above 10 nmol/l (Seki et al., 1988) and this also gives credence to a clinically relevant antagonism of carvedilol on myocardial α_1-adrenoceptors.

The rat heart has a higher cardiac α_1-adrenoceptor density than the human heart (Steinfath et al., 1992). Despite the low-receptor density, in failing human myocardium, noradrenaline evoked an α_1-adrenoceptor-mediated inotropic response of comparable size to that elicited by β-adrenoceptors (Skomedal et al., 1997). The inotropic response in human heart is also quantitatively comparable to the response observed in rat heart thus demonstrating a lack of coherence between receptor density and the size of a response mediated by the receptors.

Although speculative, the ability of carvedilol to supply a moderate blockade of myocardial α_1-adrenoceptors may reduce the chronic adrenergic drive during pathological conditions, thus attenuating the deleterious myocardial hypertrophic response to continuous neurohormonal stimulation. A moderate competitive antagonism is possible to overcome by increased adrenergic drive and in such occasions myocardial α_1-adrenoceptors might continue to provide beneficial effects as inotropic support and ischemic preconditioning.

In conclusion, carvedilol exhibits a moderate antagonism of myocardial α_1-adrenoceptors compared to that of β-adrenoceptors and this property might be one explanation for a possible additional benefit over conventional β-adrenoceptor antagonists.

Acknowledgements

We thank Line Solberg and Anne E. Sjetnan for excellent technical assistance.

This study was supported by The Norwegian Council on Cardiovascular Diseases, The Norwegian Research Council and Anders Jahre's Fund for the Promotion of Science.

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